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Olesia Priss

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Olesia Priss (Editor)

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AUTHORS

Chapter 1

Larysa Bal-Prylypko
Doctor of Technical Sciences, Professor
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0002-9489-8610

Halyna Tolok
Candidate of Technical Sciences, Associate Professor
Department of Standardization and Certification of Agricultural Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0002-2971-1645

Semen Tolok
PhD Student
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0009-0004-3023-8041

Ihor Ustymenko
Doctor of Philosophy (PhD), Associate Professor
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0003-0171-5780

Ivan Bal
Doctor of Philosophy (PhD), Associate Professor
Department of Aquaculture
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0009-0007-1608-5263

Oleksandr Kanishchev
Doctor of Philosophy (PhD), Associate Professor
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0009-0006-5034-3695

Chapter 2

Viktor Davydovych
Candidate of Veterinary Sciences
Department of Animal and Food Hygiene named after Professor A. K. Skorokhodko
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0002-2864-9018

Larysa Shevchenko
Doctor of Veterinary Sciences, Professor
Department of Animal and Food Hygiene named after Professor A. K. Skorokhodko
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0001-7472-4325

Olena Semenko
Doctor of Veterinary Sciences
Department of Parasitology and Parasitic Diseases
University Alfonso X el Sabio
ORCID: https://orcid.org/0000-0002-6453-6192

Nataliia Slobodyanyuk
Candidate of Agricultural Sciences, Professor
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0002-7724-2919

Oksana Pylypchuk
Candidate of Agricultural Sciences, Associate Professor
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0002-2757-6232

Anastasiia Ivaniuta
Candidate of Technical Sciences, Associate Professor
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0002-1770-5774

Nataliia Holembovska
Candidate of Technical Sciences,
Associate Professor
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0001-8159-4020

Alina Omelian
Candidate of Agricultural Sciences,
Assosiate Professor
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0001-9004-5250

Chapter 3

Olesia Priss
Doctor of Technical Sciences, Professor
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0000-0002-6395-4202

Iryna Ivanova
Doctor of Technical Sciences, Associate Professor
Professor Valentyna Kalytka Department of Crop Production and Horticulture
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0000-0003-2711-2021

Marina Serdyuk
Doctor of Technical Sciences, Professor
Department of Standardization and Certification of Agricultural Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0002-6504-4093

Tetiana Tymoshchuk
Candidate of Agricultural Sciences,
Associate Professor
Department of Health of Phytocenoses and Trophology
Polissia National University
ORCID: https://orcid.org/0000-0001-8980-7334

Olga Pyurko
Candidate of Biological Sciences,
Associate Professor
Department of Botany, Ecology and Park-Gardening Economy
Bogdan Khmelnitsky Melitopol State Pedagogical University
ORCID: https://orcid.org/0000-0002-3681-073X

Sergii Basanets
PhD Student
Professor Valentyna Kalytka Department of Crop Production and Horticulture
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0009-0004-6158-7367

Chapter 4

Nataliia Slobodyanyuk
Candidate of Agricultural Sciences, Professor
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0002-7724-2919

Oksana Tkachuk
PhD Student, Senior Lecturer
Department of Hotel and Restaurant Business
Odesa National University of Technology
ORCID: https://orcid.org/0000-0002-1942-0377

Tetiana Brovenko
Candidate of Technical Sciences,
Associate Professor
Department of Standardization and Certification of Agricultural Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0003-1552-2103

Tetiana Lebedenko
Doctor of Engineering Sciences, Professor
Department of Hotel and Restaurant Business
Odesa National University of Technology
ORCID: https://orcid.org/0000-0001-8385-4674

Halyna Tolok
Candidate of Technical Sciences,
Associate Professor
Department of Standardization and Certification of Agricultural Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0002-2971-1645

Tamara Novichkova
Candidate of Technical Sciences,
Associate Professor
Department of Hotel and Restaurant Business
Odesa National University of Technology
ORCID: https://orcid.org/0000-0001-6133-9882

Petro Drozd
Candidate of Historical Sciences,
Associate Professor
Department of Physiology, Plant Biochemistry and Bioenergetics
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0003-1939-2967

Mykola Gruntkovskyi
Candidate of Agricultural Sciences,
Associate Professor
Department of Animal Technology
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0002-6969-2987

Chapter 5

Oleksandr Sokot
Assistant
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0000-0003-0884-9917

Chapter 6

Nataliia Holembovska
Candidate of Technical Sciences,
Associate Professor
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0001-8159-4020

Valentyna Israelian
Candidate of Technical Sciences,
Associate Professor
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0002-7242-3227

Natalia Slobodyanyuk
Candidate of Agricultural Sciences, Professor
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0002-7724-2919

Anastasiia Ivaniuta
Candidate of Technical Sciences,
Associate Professor
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0002-1770-5774

Inna Stetsyuk
Doctor of Philosophy (PhD), Assosiate Professor
Department of Technologies of Meat, Fish and Seafood Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0001-8392-6527

Inna Kurbatova
Doctor of Biological Sciences, Professor
Department of Ecology and Zoology
Taras Shevchenko National University
ORCID: https://orcid.org/0000-0002-7333-7371

Vasyl Shynkaruk
Doctor of Philology, Professor
Department of Philosophy and International Communication
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0001-8589-4995

Yaroslav Rudyk
Candidate of Pedagogical Sciences,
Associate Professor
Department of Management and Educational Technology
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0001-5382-1505

Chapter 7

Liudmyla Kiurcheva
Candidate of Agricultural Sciences,
Associate Professor
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0000-0002-8225-3399

Mykyta Semenov
PhD Student
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0009-0001-7465-7668

Serhii Holiachuk
Candidate of Agricultural Sciences,
Associate Professor
Department of Food Technology and Chemistry
Lutsk National Technical University
ORCID: https://orcid.org/0000-0002-4835-8154

Chapter 8

Marina Serdyuk
Doctor of Technical Sciences, Professor
Department of Standardization and Certification of Agricultural Products
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0002-6504-4093

Valentyna Bandura
Doctor of Technical Sciences, Professor
Department of Processes and Equipment of Agricultural Production Processing
National University of Life and Environmental Sciences of Ukraine
ORCID: https://orcid.org/0000-0001-8074-3020

Tetiana Kolisnychenko
Candidate of Technical Sciences,
Associate Professor
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0000-0003-0560-9520

Karina Palamarek
Candidate of Technical Sciences,
Associate Professor, Head of Department
Department of Food Technology and Hotel and Restaurant Services
Chernivtsi Institute of Trade and Economics of State University of Trade and Economics
ORCID: https://orcid.org/0000-0003-4138-404X

Olha Romanovska
Candidate of Technical Sciences,
Associate Professor
Department of Food Technology and Hotel and Restaurant Services
Chernivtsi Institute of Trade and Economics of State University of Trade and Economics
ORCID: https://orcid.org/0000-0003-4027-560X

Tetiana Brykova
Candidate of Technical Sciences,
Associate Professor
Department of Food Technology and Hotel and Restaurant Services
Chernivtsi Institute of Trade and Economics of State University of Trade and Economics
ORCID: https://orcid.org/0000-0001-8842-9028

Tetiana Marusiak
Candidate of Technical Sciences,
Associate Professor
Department of Food Technology and Hotel and Restaurant Services
Chernivtsi Institute of Trade and Economics of State University of Trade and Economics
ORCID: https://orcid.org/0000-0002-1648-0016

Anastasia Parashchuk
Assistant
Department of Food Technology and Hotel and Restaurant Service
Chernivtsi Institute of Trade and Economics of State University of Trade and Economics
ORCID: https://orcid.org/0009-0002-5970-2026

Chapter 9

Nadiia Zahorko
Candidate of Technical Sciences,
Associate Professor
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0000-0003-4828-5343

Igor Dudarev
Doctor of Technical Sciences, Professor
Department of Food Technology and Chemistry
Lutsk National Technical University
ORCID: https://orcid.org/0000-0002-2016-5342

Valentyna Tkachuk
Doctor of Technical Sciences, Professor
Department of Commodity Science and Customs Expertise
Lutsk National Technical University
ORCID: https://orcid.org/0000-0001-5793-5227

Chapter 10

Olesia Priss
Doctor of Technical Sciences, Professor
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0000-0002-6395-4202

Liudmyla Kiurcheva
Candidate of Agricultural Sciences,
Associate Professor
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0000-0002-8225-3399

Pavlo Bulhakov
Doctor of Philosophy (PhD), Senior Lecturer
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0009-0002-9011-8151

Iryna Taraymovich
Candidate of Technical Sciences,
Associate Professor
Department of Food Technology and Chemistry
Lutsk National Technical University
ORCID: https://orcid.org/0000-0003-4129-2671

Larysa Chepurda
Doctor of Economic Sciences, Professor
Department of Tourism and Hotel and Restaurant Business
Cherkasy State Technological University
ORCID: https://orcid.org/0000-0002-8941-9427

Sergii Stepanenko
Candidate of Economic Sciences
Department of Chemistry, Biochemistry, Microbiology and Food Hygiene
State Biotechnological University
ORCID: https://orcid.org/0009-0009-4655-9110

Chapter 11

Ludmila Romanovich
Assistant
Department of Normal and Pathological Physiology named after Stepan Stoyanovsky
Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies
ORCID: https://orcid.org/0000-0002-4546-5210

Bohdan Kurtyak
Doctor of Veterinary Sciences,
Head of Department
Department of Epizootology
Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies
ORCID: https://orcid.org/0000-0001-8401-5928

Olena Danchenko
Doctor of Agricultural Sciences, Professor
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0000-0001-5049-3446

Chapter 12

Sergiy Smirnov
PhD Student
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0009-0005-9083-2496

Olesia Priss
Doctor of Technical Sciences, Professor
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0000-0002-6395-4202

Svitlana Danylenko
Doctor of Technical Sciences, Professor
Department of Biotechnology
Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine
ORCID: https://orcid.org/0000-0003-4470-4643

Chapter 13

Olesia Priss
Doctor of Technical Sciences, Professor
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0000-0002-6395-4202

Yanina Chetverikova
Assistant
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0009-0007-5359-864X

Viktoriia Vertegel
Candidate of Pedagogical Sciences
Department of Foreign Languages for Professional Purposes
Zaporizhzhia National University
Department of Foreign Languages
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0000-0002-5100-3784

Chapter 14

Igor Dudarev
Doctor of Technical Sciences, Professor
Department of Food Technology and Chemistry
Lutsk National Technical University
ORCID: https://orcid.org/0000-0002-2016-5342

Tamara Sydoruk
PhD Student
Department of Food Technology and Chemistry
Lutsk National Technical University
ORCID: https://orcid.org/0009-0006-9008-9556

Mykola Andrushchenko
Candidate of Agricultural Sciences, Associate Professor
Department of Food Technology and Hotel and Restaurant Business
Dmytro Motornyi Tavria State Agrotechnological University
ORCID: https://orcid.org/0000-0002-7233-4339

ABSTRACT

Innovative technologies of dairy-plant-based products for functional purposes. The development of innovative dairy-plant-based products for functional purposes is a promising direction in modern food technology. Recipe optimization involved the incorporation of soy-fat concentrate, chia, quinoa, and flax seeds as sources of polyunsaturated fatty acids, dietary fiber, and antioxidants, as well as dihydroquercetin to enhance antioxidant stability and extend shelf life. The proposed approach ensured the formation of a balanced protein-lipid composition, improvement of the fatty acid profile, and enrichment with biologically active compounds, resulting in increased antioxidant potential.

Monitoring of acarid mite populations in craft hard goat cheeses during ripening. The use of mites in the ripening of artisanal hard goat cheeses contributes to distinctive sensory characteristics but requires safety assessment. A methodology for monitoring *Acarus siro* density was developed. For unwashed cheeses, mites are determined in surface powder per unit mass, whereas for washed cheeses, they are counted in rind scrapings per unit area. Treatment with linseed oil eliminated *Acarus siro* mites and eggs but promoted secondary mold growth.

The effect of protective composition on the quality of sweet cherry during storage. The effect of an exogenous protective composition based on lactic and acetic acids on the preservation of sweet cherry fruits during refrigerated storage was studied. Sweet cherry fruits of nine varieties were pre-cooled using air cooling, hydrocooling, and a combined method involving hydrocooling in organic acid solutions followed by air cooling, and stored. The highest yield of marketable fruits was obtained using combined cooling with the protective composition. This treatment reduced oxidative stress, as evidenced by lower malondialdehyde content and balanced antioxidant enzyme activity.

Evaluation of the usage of spontaneous fermentation sourdough starters and their influence on the quality indicators of wheat bread. The feasibility of using spontaneous fermentation sourdoughs (dry and liquid starters based on wine yeast) in wheat bread production was evaluated. Physical, chemical, biotechnological, and sensory properties of the starters were determined, and test baking was conducted. Bread with 5% and 7% sourdough was compared with control samples. The addition of sourdough increased specific volume and porosity, slowed moisture loss, and improved sensory characteristics.

Technological and qualitative aspects of enriching wheat bread with oyster mushroom paste. The feasibility of using oyster mushroom (Pleurotus ostreatus)

paste as a functional ingredient in wheat bread was investigated. By-products of mushroom processing were proposed to reduce costs and improve economic efficiency. Bread with 10–30% mushroom paste was evaluated for physicochemical properties. The addition of mushroom paste caused moderate changes in bread characteristics and amino acid composition without exceeding quality standards, confirming its potential for functional bakery products.

Improvement of technology of fish pastes with the addition of non-traditional raw materials. The study investigated the use of goji berries as a functional additive in crucian carp fish sticks. The addition of berries increased mineral content, reduced moisture, and improved sensory and textural properties. During storage at 4°C for 4 days, the samples retained physicochemical stability and high sensory quality. The results confirm the feasibility of using goji berries to improve the nutritional and functional value of fish products.

Innovative approaches to the storage technology of dehydrated meat semi-finished products using natural antioxidants. The effect of convective drying and treatment with a natural antioxidant (trans-ferulic acid) on the stability of the structure and preservation of nutritional value of semi-finished meat products from chicken and pork was studied. The optimal parameters of the technology for extending shelf life were determined.

Development of sauce technology from fermented plant-based materials for the food industry and HoReCa. The study focused on developing a technology for sauces based on fermented plant materials (legumes, grains, pseudo-grains, and vegetables) using *Lactiplantibacillus plantarum*. Fermentation led to a decrease in pH, carbohydrates, and sugars, along with a shift in redox potential, indicating improved functional properties of the raw materials. The resulting sauces exhibited lower energy value, stable consistency, and distinctive flavor profiles compared to the control (boiled chickpea-based sauce).

Impact of long-term storage on the quality of frozen pickled sweet peppers. This study investigated frozen pickled sweet peppers prepared with a marinade of oil, sugar, honey, salt, citric acid, and spices, followed by storage at −20°C for 270 days. Moderate losses of ascorbic acid, carotenoids, dry matter, and sugars were observed, while some bioactive compounds increased. Freezing improved microbiological safety and maintained high sensory quality. Microwave thawing was recommended, although prolonged storage after thawing reduces quality despite remaining within safety limits.

Effect of a combined biopolymer coating on the quality of asparagus spears during storage. The effect of a biopolymer coating based on sodium alginate and the antioxidant rutin on the quality of asparagus during storage was studied. The effect

of the coating on the preservation of the marketable, physiological and organoleptic characteristics of asparagus was considered. The use of the studied biopolymer allowed to slow down respiratory metabolism, degradation of chlorophylls and carotenoids and stabilize the organoleptic characteristics of the product.

Fatty acid composition of total lipids of liver and thigh muscle broiler chickens under the influence of separate and complex action of vitamins E and C. The study investigated the effect of vitamins E and C, applied separately and in combination, on the fatty acid composition of liver and skeletal muscle lipids in 41-day-old broiler chickens. Supplementation with vitamin E increased certain saturated fatty acids and ω-3 PUFA, while vitamin C led to a rise in saturated fatty acids and a decrease in PUFA, particularly ω-6. The combined use of vitamins E and C caused the most pronounced changes, including a significant increase in total PUFA and ω-6, along with a higher ω-6/ω-3 ratio. In muscle tissues, vitamin E improved the balance of PUFA and enhanced the biological value of meat, whereas vitamin C increased antioxidant activity but maintained the fatty acid ratio at the control level.

Microbiological stability of filled gingerbread: problems and technological solutions. This study combines a literature review and a case study to identify factors influencing spoilage and effective strategies for controlling microbiological stability. Differences in composition between the crumb and filling promote moisture migration and localized increases in water activity, creating favorable conditions for microbial growth, while post-baking contamination also plays a significant role. The results highlight the need for a comprehensive "hurdle" approach, including control of ingredient aw, proper hygienic practices, barrier packaging, and formulation optimization to enhance the microbiological stability of the product.

Kale as a functional vegetable. Nutritional value, bioactive compounds and the influence of processing and cultivation. Kale *(Brassica oleracea var. acephala)* is a nutrient-rich leafy vegetable with high levels of glucosinolates, isothiocyanates, and phenolic compounds that exhibit antioxidant and health-promoting properties. This review summarizes factors affecting their accumulation, including biotic and abiotic stresses, as well as the impact of processing methods. Water-based heat treatment reduces bioactive compounds, while steaming, short frying, and freezing better preserve them; non-thermal technologies also show potential for enhancing their bioavailability. The findings emphasize the importance of optimized cultivation and processing conditions, confirming kale as a promising raw material for functional foods.

Frozen desserts formulated with plant-based milk: a comprehensive quality analysis. The consumption of plant-based frozen desserts is increasing, driven by the use of alternative milks such as soy, coconut, rice, and others, which enhance products with bioactive compounds. This study analyzes their nutritional and physicochemical

properties, noting variability depending on formulation and processing, while emphasizing the need to match conventional ice cream quality. Key quality indicators were systematized, and a comprehensive quality index was developed for objective product evaluation. SWOT analysis enabled the identification of strengths, weaknesses, and development strategies, supporting improved formulation and market positioning of plant-based frozen desserts.

Keywords

Functional foods, healthy nutrition, biologically active compounds, dairy-plant products, curd-based products, bread, bakery products, broiler chicken meat, meat semi-finished products, fish sticks, sauces, pickled vegetables, kale, asparagus, sweet cherry, sweet pepper, processing, cooling, freezing, dehydration, convective drying, recipe optimization, organoleptic properties, physicochemical properties, fatty acid composition, water activity, microbiological spoilage, antioxidants, phenolic compounds, vitamin E, vitamin C, storage, shelf life, postharvest treatment, postharvest losses, oxidative stress.

CIRCLE OF READERS AND SCOPE OF APPLICATION

The primary audience for this volume is academic and R&D researchers working in food science and technology, food chemistry, microbiology, and applied nutrition. This collection brings together research-based innovations and practical solutions in food technology, exploring challenges that shape the field and spanning a wide range of products. Although every specialist will inevitably find some chapters more directly relevant to their work than others, the volume presents an insightful cross-section of facts and methodologies, offering inspiration for new approaches and research topics.

Food technologists employed in industrial or small-scale production will recognize the practical concerns that motivate several of the studies here – questions of shelf life, microbiological stability, the behavior of enriched formulations under real storage conditions, the economics of incorporating novel ingredients. The chapters on filled gingerbread spoilage, dehydrated meat products, and frozen pickled peppers are grounded in precisely the kind of quality assurance problems that arise routinely in production settings, unveiling the potential of applied technology improvements based on scientific analysis of product quality and safety. Moreover, the chapters, focused on the food fortification, offer innovative solutions for managers and entrepreneurs, aiming for the cost-effective and sustainable productions of products with high nutrition value.

Detailed monitoring of bioactive compounds, conducted across experiments on fresh and stored plant products, fish paste, and broiler chicken, may also be of interest to veterinarians and specialists working in clinical nutrition and public health research – particularly in areas such as lipid metabolism, dietary antioxidants, and the health implications of plant-based diets. Professionals in the HoReCa sector, especially those involved in menu development, product sourcing, or the growing segment of functional and plant-forward food service, may find this information relevant for practical decision-making in the context of health-oriented food products.

Lastly, the volume may serve as a supplementary resource for university courses in food technology, applied biochemistry, or agricultural sciences, reflecting current trends of these industries and outlining their future perspectives.

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INTRODUCTION

Food science at a crossroads: innovation, functionality, and the future of food technology

The modern food technology landscape is broad, technically demanding, and still expanding. Although this discipline has never been static, the pace and character of its current evolution seem to differ qualitatively from previous decades. The primary tasks of product preservation, safety, and shelf life have expanded rapidly into areas that would have seemed peripheral to earlier generations of food scientists. Approaches that enable targeted modulation of nutritional profiles, the rational use of fermentation to improve functionality, post-harvest management of bioactive compounds, and the design of products for specific physiological outcomes are no longer niche research topics – they now define the core of the field.

The chapters collected in this volume reflect that shift. They do not follow a single unified theory or rely on a common method; rather, they present results from independent research groups working on diverse problems. These include the enrichment of dairy-plant formulations with polyunsaturated fatty acids, the sensory and safety dimensions of mite-ripened artisanal goat cheeses, postharvest treatments for sweet cherry and asparagus, sourdough fermentation in wheat bread, the incorporation of oyster mushroom paste and goji berries into conventionally defined product categories, the stability of frozen and dehydrated preparations, and the emerging science of plant-based frozen desserts. What connects these studies is a shared orientation: a willingness to interrogate existing formulations and technologies, and to ask how they can be improved – nutritionally, functionally, economically, or in terms of consumer acceptability.

A few chapters here provide bioactive compound profiling and analyze biochemical shifts under different cultivation, processing and preservation conditions – data, critically necessary for systematic approach to improving product shelf life and safety. In the chapter on frozen plant-based desserts empirical results the physicochemical analysis is combined with a SWOT-based assessment of market positioning – a methodological choice that reflects the practical pressures under which much contemporary food research is conducted.

Similarly, several of the contributions engage, in different ways, with the tension between novelty and standardization. Introducing mushroom paste into bread,

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or fermented legume sauces into the HoReCa supply chain, is not merely a matter of adding an ingredient; it requires demonstrating that quality parameters remain within acceptable limits, that the technological process is reproducible, and that the resulting product can be positioned meaningfully within existing regulatory and market frameworks. This type of work is often overlooked in favor of more visible, high-profile innovations. However, it is careful, empirically grounded effort that makes new formulations viable in practice, and therefore several chapters in this volume are dedicated for such an applied research.

The attention paid to by-product valorization and resource efficiency across several chapters deserves particular mention. The use of mushroom processing residues, the application of organic acid solutions in hydrocooling, the development of fermented sauces from legumes and pseudo-grains – these are not merely cost-reduction strategies. They reflect a broader rethinking of what counts as a raw material, and a growing awareness that the food industry's environmental footprint is itself a legitimate object of technological intervention. Whether this constitutes a coherent paradigm shift or a collection of pragmatic responses to economic and regulatory pressure is a question the reader may wish to consider.

The editors thank the contributing authors for their work and for their patience with the editorial process. This volume makes no claim to comprehensiveness; the field is too large and too rapidly evolving for any single collection to capture it fully. What it does offer is a cross-section of current research that is, we believe, representative of the directions in which food technology is moving – and of the rigor with which that movement is being documented.

CHAPTER 1

Innovative technologies of fermented dairy-plant-based products for functional purposes

Larysa Bal-Prylypko
Halyna Tolok
Semen Tolok
Ihor Ustymenko
Ivan Bal
Oleksandr Kanishchev

Abstract

The development of innovative technologies for functional dairy products, as well as the improvement of traditional formulations in accordance with modern healthy nutrition requirements, represents one of the priority directions in the advancement of food science and industry. In the context of the increasing prevalence of diet-related diseases, the creation of next-generation products with enhanced biological and physiological value is of particular importance.

A significant share of functional products consists of enriched items formulated with natural additives in various physical forms, including powders, extracts, concentrates, and microencapsulated ingredients. Their application contributes to compensating for essential nutrient deficiencies, optimizing fatty acid composition, increasing the antioxidant potential of the diet, and strengthening the body's resistance to adverse environmental factors.

Fermented dairy products, particularly cottage cheese and curd-based products, are traditionally perceived by consumers as components of a balanced diet due to their optimal ratio of proteins, fats, and carbohydrates, high digestibility, and the presence of complete milk proteins. However, their vitamin-mineral composition and profile of biologically active compounds do not always fully comply with contemporary concepts of functional nutrition, which necessitates technological modernization through the incorporation of ingredients with pronounced physiological effects.

A promising approach to addressing this issue is the development of fermented dairy-plant-based products with a combined protein and lipid composition. Recipe

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optimization involves the use of soy-fat concentrate to improve the fatty acid profile and increase the proportion of polyunsaturated fatty acids; chia, quinoa, and flax seeds as natural structure-forming agents and sources of dietary fiber, omega-3 fatty acids, phenolic compounds, and antioxidants; and dihydroquercetin as a natural antioxidant and preservative that enhances oxidative stability and extends shelf life.

The absence of gluten in the investigated plant raw materials creates opportunities for the development of functional curd-based products suitable for individuals with celiac disease and gluten intolerance.

Thus, the integration of traditional curd production technology with modern approaches to functional ingredient incorporation makes it possible to expand the range of domestic dairy-plant products with health-promoting properties, enhance their competitiveness, and adapt them to current consumer demands.

Keywords

Functional foods, curd product, soy-fat concentrate, chia seeds, quinoa, flax seeds, dihydroquercetin, quality, safety, nutritional value, biological value.

1.1 Introduction

The development of new technologies for functional dairy-plant-based products and the improvement of existing ones is an extremely relevant task of modern food science.

Functional foods are aimed at maintaining and strengthening human health, providing the body with essential biologically active substances.

At the present stage of food industry development, the market of functional products is mainly focused on segments related to supporting human health, in particular the cardiovascular and digestive systems, as well as body weight management and strengthening of bone tissue [1].

The largest share among functional foods is represented by fortified products enriched with vitamins, micronutrients, dietary fiber, plant proteins, and other biologically active components. The use of natural additives in various physical forms makes it possible to compensate for deficiencies of essential nutrients and to increase the body's nonspecific resistance to adverse environmental factors [2].

For a long time, fermented dairy products, particularly cottage cheese and products based on it, have been perceived by consumers as being close to a rationally balanced diet, since they provide an optimal ratio of energy-significant nutrients – proteins, fats, and carbohydrates. At the same time, their consumer, vitamin, and mineral characteristics do not fully meet modern requirements of healthy nutrition, which

necessitates their improvement through the incorporation of new components with specific physiological effects [3].

To address this issue, the development of new and the improvement of existing technologies for producing fermented dairy-plant-based products using cottage cheese as a base, in particular cheese-based desserts (curd products), is considered promising. The use of emulsion-type fat concentrates to completely replace milk fat with vegetable oils will not only allow targeted correction of the fatty acid composition of curd products and enrichment with biologically active lipid complexes of plant origin, but will also help solve the problem of insufficient milk raw materials and seasonal variations in their chemical composition [4].

In order to increase biological value and impart functional properties to curd products, it is advisable to use chia, quinoa, and flax seeds.

1.2 Justification for the use of raw materials and ingredients to improve the technology of a curd product

The need to combine food products is driven not only by current challenges in the food industry (shortage of high-quality raw materials, their insufficient quality, and incomplete utilization of all constituent components), but also by the necessity to provide consumers with a functional diet against the background of inadequate intake of protein, vitamins, macro- and microelements, and other essential nutrients.

When developing composite mixtures, a comprehensive approach should be followed, taking into account several key requirements: plant-based additives should not only exert a positive effect on biological and physiological processes in the human body, but also be economically feasible, available in the required quantities, consistent in quality, and easily integrated into the technological production process.

1.2.1 Justification for the use of an emulsion concentrate based on corn oil in the curd product formulation

Single-type or blended vegetable oils are widely used in technologies for dairy-plant-based products, particularly in the form of food emulsions, as lipid enrichers, functional ingredients, and structure-forming agents of the fat phase [5–7].

The content of tocopherols is an important indicator of both the biological value of products and their oxidative stability. Natural tocopherols are represented

by four structural isomers: α-, β-, γ-, and δ-tocopherols. Although all isoforms are biologically active, α-tocopherol exhibits the highest biological activity in the human body. At the same time, γ- and δ-tocopherols play a leading role in providing antioxidant protection of lipids directly in oils and fat-containing products [8].

Tocopherols are capable of inhibiting lipid peroxidation processes, inactivating free radicals, and interrupting chain reactions of auto-oxidation, thereby increasing oil stability during storage. Due to this, they perform a dual function: on the one hand, they enhance the biological value of functional food products; on the other hand, they contribute to extending their shelf life [9].

The total tocopherol content in refined corn oil is significantly higher compared to other types of vegetable oils (**Table 1.1**), which constitutes a strong argument for selecting this oil for use in the soy-fat concentrate formulation.

Table 1.1 Tocopherol content in corn oil compared with other types of oils

Type of oil	Tocopherol content, mg/kg			Total tocopherols
	α	$(\beta+\gamma)$	δ	
Sunflower	490 ± 24.50	30.8 ± 1.54	10.1 ± 0.50	535 ± 26.75
Corn	207 ± 11.0	592 ± 30.0	30.0 ± 1.50	829 ± 41.45
Rapeseed	162 ± 8.10	174 ± 8.7	136 ± 6.8	472 ± 23.6
Olive	163 ± 38.15	12.3 ± 0.62	1.6 ± 0.08	177 ± 8.85

Source: [9]

Taking this into account, the authors of this scientific work developed a soy-fat concentrate based on corn oil, which represents a food emulsion [10]. The formulation of the soy-fat concentrate is presented in **Table 1.2**.

Table 1.2 Formulation of the soy-fat concentrate

Raw material	Content, %
Refined deodorized corn oil	49.00
Soy milk powder	4.50
Sodium caseinate	0.70
Emulsifier	0.50
Dihydroquercetin	0.012
Drinking water	45.288

Source: [10]

The soy-fat concentrate is characterized by high organoleptic quality indicators and functional properties due to the use of dihydroquercetin, which acts on free radicals by neutralizing them, thereby suppressing the development of various diseases [11]. Dihydroquercetin also exhibits other health-promoting properties, including a positive effect on the cardiovascular system [12].

Dihydroquercetin demonstrates significantly higher antioxidant activity compared to certain other antioxidants. In particular, its antioxidant activity exceeds that of quercetin by 92.412%, vitamin C by 101.062%, and vitamin E by 101.992% [13]. This makes it possible to state that dihydroquercetin is one of the most potent natural antioxidants, and its inclusion in the soy-fat concentrate enables the production of a curd product with functional properties.

1.2.2 Justification for the use of plant-based enrichers in the curd product formulation

In classical technologies for curd spreads, a stabilizer or stabilization system is an essential formulation component. As a rule, multiple binding of free moisture – the main technological effect of such additives – is characteristic of a wide range of polysaccharides (pectin, starch and its chemically modified forms, agar, alginate, carrageenan, gums, etc.), as well as certain protein compounds such as gelatin and milk protein concentrates. Such substances, which are relatively expensive, are typically imported and used according to manufacturers' recommendations in amounts ranging from 0.2% to 1.5% [14]. Therefore, the authors of this scientific work propose reducing the use of synthetic stabilizers in curd product production by applying plant-based raw materials containing gums and mucilaginous substances. These components are capable of improving the rheological properties of mixtures and contributing to the formation of the desired texture. An additional advantage of using plant additives is the enrichment of food products with dietary fiber and other ballast substances, which positively affects their functional value [14].

Thus, a scientific interest lies in analyzing the composition of seeds and pseudocereals such as chia, quinoa, and flax.

The study revealed that the physicochemical and technological properties of seeds largely depend on numerous factors, including varietal characteristics, soil and climatic conditions, cultivation technology, and maturity stage. The macronutrient content and energy value of the studied seed samples are presented in **Table 1.3**. The data in **Table 1.3** indicate that chia, quinoa, and flax seeds are characterized by a low carbohydrate content and an increased content of proteins, lipids, and dietary fiber.

Table 1.3 Comparative characteristics of the chemical composition of seeds

Indicator	Chia	Quinoa	Flax
Protein content (g/100 g dry matter)	15.9 ± 0.08	13.62 ± 0.07	17.8 ± 0.09
Lipid content (g/100 g dry matter)	30.7 ± 0.16	5.92 ± 0.03	41.6 ± 0.21
Carbohydrate content (g/100 g dry matter)	42.8 ± 0.22	67.21 ± 0.34	29.1 ± 0.15
Dietary fiber (g/100 g dry matter)	34.7 ± 0.18	7.4 ± 0.04	27.1 ± 0.14
Energy value (kcal/100 g)	511 ± 2.56	376.60 ± 1.88	562 ± 2.81

Source: [9, 14]

Thus, the protein content in seeds may vary approximately from 14% to 18%, depending on the seed type as well as cultivation conditions, temperature, and humidity. Seed proteins demonstrate good digestibility (78.9%), which is comparable to casein (88.6%) and higher than that of proteins contained in corn (66.6%), rice (59.4%), wheat (52.7%), and even amaranth (90%) [15].

Seeds and pseudocereals are often incorporated into food products to meet consumer demand for gluten-free products. In recent years, this market has shown exponential growth due to the increasing prevalence of gluten-related disorders (celiac disease), non-celiac gluten sensitivity, and wheat allergy, as well as rising demand among population groups that choose a gluten-free diet because it is perceived as healthier [16]. These facts further confirm the feasibility of using seeds as gluten-free enriching ingredients for the development of products with enhanced biological value.

The amino acid composition of proteins from chia, quinoa, and flax seeds is presented in **Table 1.4**.

The analysis of the amino acid composition confirmed the presence of 10 essential amino acids, among which the highest levels were observed for arginine, leucine, phenylalanine, valine, and lysine. Seed proteins are also rich in non-essential amino acids, mainly cystine, tyrosine, and alanine. The differences in the content of individual amino acids between the seeds were not statistically significant ($P < 0.05$). The proportion of essential amino acids relative to the total amino acid content – considered an indicator of protein quality – was 37.87%, 33.76%, and 35.18% for chia, flax, and quinoa seeds, respectively, indicating the high biological quality of these proteins [19].

Particular interest is associated with the fatty acid profile. It is characterized by a high content of polyunsaturated fatty acids (PUFAs), mainly α-linolenic acid (ALA), which accounts for approximately 60% of total fatty acids (**Table 1.5**).

Table 1.4 Content of essential amino acids in seeds, g/100 g

Amino acid	Chia	Quinoa	Flax
Arginine	2.14	0.758	1.92
Histidine	0.53	0.033	0.47
Isoleucine	0.80	0.404	0.90
Leucine	1.37	0.561	1.24
Lysine	0.97	0.480	0.86
Methionine	0.59	5.360	0.37
Phenylalanine	1.02	0.253	0.96
Threonine	0.71	0.071	0.77
Tryptophan	0.44	0.38	0.29
Valine	0.95	0.571	1.07
Cystine	0.41	0.35	0.34
Tyrosine	0.56	0.130	0.49
Alanine	1.04	0.654	0.93

Source: [17, 18]

Table 1.5 Fatty acid content in seeds, g/100 g

Fatty acids	Chia	Quinoa	Flax
Saturated fatty acids (SFAs)			
Palmitic acid (C16:0)	7.1	–	2.17
Stearic acid (C18:0)	3.24	1.03	1.33
Monounsaturated fatty acids (MUFAs)			
Palmitoleic acid (C16:1)	0.2	–	0.09
Oleic acid (C18:1 – ω-9)	10.53	27.6	7.36
Eicosenoic acid (20:1)	0.16	–	0.07
Polyunsaturated fatty acids (PUFAs)			
Linoleic acid (C18:2 – ω-6)	20.37	5.7	5.9
Linolenic acid (C18:3 – ω-3)	59.76	54.9	58.2
Eicosadienoic acid (20:2)	0.08	–	0.07
Total PUFAs	80.4	73.63	60.6
Omega-6/Omega-3 ratio	0.35	0.30	0.32

Source: [17, 19]

Linoleic, oleic, and palmitic acids are present in smaller amounts. Chia seeds contain a higher level of omega-3 fatty acids compared to flax seeds. In general, chia seeds contain several times more fat than cereal grains, with particularly high

levels of omega-3 fatty acids, including 41–59% α-linolenic acid (omega-3) and 18–25% linoleic acid (omega-6).

Compared with other products traditionally considered rich in omega-3 fatty acids, the amount of these fatty acids in chia seeds is almost twice that found in salmon roe, three times higher than in cod liver, and 42 times higher than in olive oil. The average omega-3 fatty acid content in chia seeds is about 21%, while in flax seeds it is approximately 17% [20].

Another valuable characteristic of chia, quinoa, and flax additives is their dietary fiber content. Dietary fibers – such as cellulose, pectin, and hemicellulose – contribute to reducing the caloric density of the diet, mitigating the negative impact of excessive fat and carbohydrate consumption on metabolic processes, and regulating intestinal motility. Dietary fibers are also capable of adsorbing and removing various chemical substances, including carcinogens, from the human body.

The presence of chia, quinoa, and flax seeds in curd products may significantly influence microbial growth. On the one hand, due to the presence of antimicrobial compounds, they may reduce total microbial contamination; on the other hand, microbial populations present on the seed surface may increase it. For example, chia seeds contain phenolic compounds (**Table 1.6**) [21].

Table 1.6 Phenolic compound content in chia and quinoa seeds, mg/g

Phenolic compounds	Chia	Quinoa
Chlorogenic acid	0.10–0.23	0.42–0.63
Myricetin	0.11–0.12	0.14–0.19
Quercetin	0.15–0.27	0.19–0.39
Kaempferol	0.36–0.50	0.14–0.21

Source: [22, 23]

Chia, quinoa, and flax seeds also contain natural tocopherols, which increases their value for use in the technology of a new type of curd product (**Table 1.7**). The presence of α-, γ-, and δ-tocopherols allows for a natural increase in the oxidative stability of the fat phase without the addition of synthetic antioxidants [17].

As shown in **Table 1.7**, the highest tocopherol content is observed in chia and flax seeds, while somewhat lower levels are found in quinoa seed lipids. β + γ-tocopherols account for 72–90% of the total tocopherols in flax seeds, 71–75% in quinoa seeds, and 62–92% in chia seeds. This confirms the hypothesis that these seeds possess high biological value and may serve as a potential source of nutraceutical lipids capable of enriching curd products with functional components.

Table 1.7 Tocopherol content in seeds (mg/kg of oil)

Seed type	Tocopherol content, mg/kg			Total tocopherols	Profile characteristics
	α	$(\beta + \gamma)$	δ		
Chia	7–15	400–600	10–25	510–650	γ-tocopherol predominates – high antioxidant activity
Quinoa	40–90	150–300	5–15	200–420	α- and γ-forms predominate
Flax	10–25	300–500	15–35	430–560	High γ and δ content

Source: [17]

Thus, the combination of a favorable amino acid composition, a high content of polyunsaturated fatty acids, dietary fiber, phenolic compounds, and natural tocopherols substantiates the feasibility of using chia, quinoa, and flax seeds in the formulation of functional curd products with enhanced biological value and improved technological properties.

1.2.3 Justification for the use of sea salt in the curd product formulation

To impart a savory character to the curd product, the inclusion of salt in the formulation has been proposed. However, classical curd paste technologies typically use table salt (sodium chloride), and it has been demonstrated [24] that excessive sodium intake contributes to an increased risk of various diseases. Therefore, in order to reduce sodium intake, the use of sea salt is considered relevant. The feasibility of incorporating sea salt into the diet can also be justified from a nutritional perspective, as dietitians recommend reducing sodium content while maintaining the required mass fraction of chloride. In addition, sea salt allows the enrichment of the finished product with essential minerals, particularly potassium, thereby increasing its nutritional value [25].

1.3 Development of the technology for producing a new-generation curd product

1.3.1 Justification of the protein-fat base composition

Initially, the composition of the protein-fat base was substantiated for its subsequent enrichment with plant components to obtain the finished product. Low-fat

cottage cheese produced by the acid-rennet method was used as the milk-protein base, in accordance with the requirements of DSTU 4554:2008 "Cottage Cheese. Specifications".

Experimental formulations of the protein-fat base with a specified fat content are presented in **Table 1.8**.

The organoleptic characteristics of the protein-fat base samples compared with the control sample are presented in **Table 1.9**.

Table 1.8 Experimental formulations of the protein-fat base

Formulation components	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4
	Corn oil content, %			
	7.0	8.0	9.0	10.0
Low-fat cottage cheese	86.0	84.0	82.0	80.0
Soy-fat concentrate	14.0	16.0	18.0	20.0
Total	100.0	100.0	100.0	100.0

Table 1.9 Organoleptic characteristics of protein-fat base samples

Indicators	Sample				
	Control	No. 1	No. 2	No. 3	No. 4
Consistency, appearance	Crumbly, slight graininess		Slightly softened, spreadable	Excessively softened, non-uniform	
Color	White, uniform throughout			Creamy shade, uniform throughout	
Taste and odor	Characteristic fermented milk flavor, without foreign tastes and odors			Fermented milk flavor with a slight corn oil aftertaste	

According to **Table 1.9**, samples with different fat contents differ significantly in their organoleptic characteristics. As the fat content increases from 7% to 10%, the consistency of the protein-fat base becomes softer and more spreadable, with a more oily texture. The taste acquires a slightly sweet and somewhat bland aftertaste. A significant loss of product homogeneity was observed at a corn oil content above 8%.

The physicochemical parameters of the protein-fat base samples are presented in **Table 1.10**.

According to **Table 1.10**, as the fat content in the protein-fat base increases from 7% to 10%, titratable acidity decreases, reaching nearly half of its initial value. In contrast, active acidity (pH) changes within a narrower range. Despite the relatively high water content in the soy-fat concentrate, increasing the fat content of the samples

leads to a decrease in overall moisture against the background of slight softening of consistency. At the same time, the water-holding capacity of samples with different fat contents remains almost identical, which can be explained by the presence of an effective hydrophilic emulsifier – sodium caseinate – in the aqueous phase of the soy-fat concentrate.

Table 1.10 Physicochemical parameters of protein-fat base samples

Indicator	Control / No. 1	Control / No. 2	Control / No. 3	Control / No. 4
Titratable acidity, °T	166.5 ± 3.2 / 164.8 ± 2.9	135.0 ± 2.2 / 132.0 ± 1.9	113.5 ± 3.3 / 109.3 ± 2.4	109.0 ± 2.9 / 96.5 ± 2.7
Active acidity, pH	4.38 ± 0.10 / 4.35 ± 0.09	4.41 ± 0.10 / 4.41 ± 0.19	4.50 ± 0.10 / 4.45 ± 0.08	4.60 ± 0.10 / 4.59 ± 0.09
Moisture content, %	79.3 ± 1.9 / 79.1 ± 1.5	77.2 ± 1.5 / 77.1 ± 1.5	74.2 ± 1.4 / 74.5 ± 1.7	70.1 ± 2.0 / 69.4 ± 1.2
Water-holding capacity, %	70.4 ± 1.9 / 67.8 ± 1.7	72.4 ± 3.1 / 67.1 ± 2.8	74.4 ± 2.6 / 66.8 ± 2.0	73.4 ± 3.3 / 65.4 ± 2.7

Thus, based on the assessment of organoleptic and physicochemical parameters, the feasibility of using the soy-fat concentrate in the protein-fat base has been established, with a recommended fat content range of up to 8.0% for further application in the curd product formulation.

1.3.2 Justification of the degree of grinding of chia, quinoa, and flax seeds prior to incorporation into the protein-fat base

From a technological standpoint, the structure of curd products, particularly combined-composition curd spreads, is one of the key quality indicators. Inconsistencies in their rheological characteristics may lead to an increased proportion of defective products unsuitable for further packaging, storage, and distribution.

To substantiate the feasibility of using plant raw materials in the curd product formulation, a comparative analysis of the structural and mechanical properties of whole and ground seeds was carried out. The following fractions were studied: sample No. 1 (≤ 1.0 mm), sample No. 2 (≤ 0.7 mm), and sample No. 3 (≤ 0.5 mm).

The research results demonstrated that the water-holding capacity (WHC) of the samples is influenced by several factors:

– degree of grinding – smaller particle sizes exhibit significantly higher WHC values;

– hydration temperature – the optimal range is 40–60°C;

– medium pH – the most favorable pH values are 4–5;

– hydration time – the optimal duration is 30–60 minutes.

The results of the study on the water-holding capacity of whole and ground chia, quinoa, and flax seeds are presented in **Table 1.11**.

Table 1.11 Water-holding capacity of whole and ground chia, quinoa, and flax seeds

Seed type	Water-holding capacity (W), %			
	Whole seeds	Sample No. 1 (≤ 1.0 mm)	Sample No. 2 (≤ 0.7 mm)	Sample No. 3 (≤ 0.5 mm)
Chia	820 ± 41	1000 ± 50	1250 ± 63	1470 ± 74
Quinoa	160 ± 8	220 ± 11	280 ± 14	310 ± 16
Flax	340 ± 17	510 ± 26	600 ± 30	750 ± 38

The data presented in **Table 1.11** correlate with the chemical composition of the seeds. In particular, chia seeds contain the highest level of dietary fiber (34.7 ± 0.18 g/100 g dry matter), which explains their superior water-holding capacity (820–1470%). Upon hydration, a gel (dense colloidal system) is formed that can retain large amounts of water even without heat treatment. Quinoa seeds contain approximately 7.4% fiber; therefore, their WHC is lower than that of chia seeds. However, this parameter may increase after thermal processing or enzymatic modification.

The water-holding capacity of flax seeds (340–750%) is determined by the presence of mucilage substances (a mixture of homo- and heteropolysaccharides and polyuronides that easily swell in water), cellulose, hemicellulose, and proteins. Flaxseed mucilage contains fibrous materials (18–45 nm in diameter) that expand in the presence of water [26].

The relationship between water-holding capacity and hydration time for whole and ground seeds is shown in the **Fig. 1.1.**

Seeds contain a significant amount of water-soluble carbohydrate substances, the presence of which determines the degree of swelling and mass increase in all experimental samples (**Fig. 1.1**). Viscous solutions are formed during hydration. It was observed that the amount of gel formed increased in ground seeds compared to whole seeds for all three types. The swelling process of both whole and ground chia seeds occurs most intensively during the first 30 minutes, followed by stabilization of the indicators over the next 30 minutes. The swelling behavior of flax and quinoa seeds is more uniform over time; however, they differ in the amount of gel formed during the study. The lowest swelling indices were observed in quinoa seed samples due to their lower fiber content.

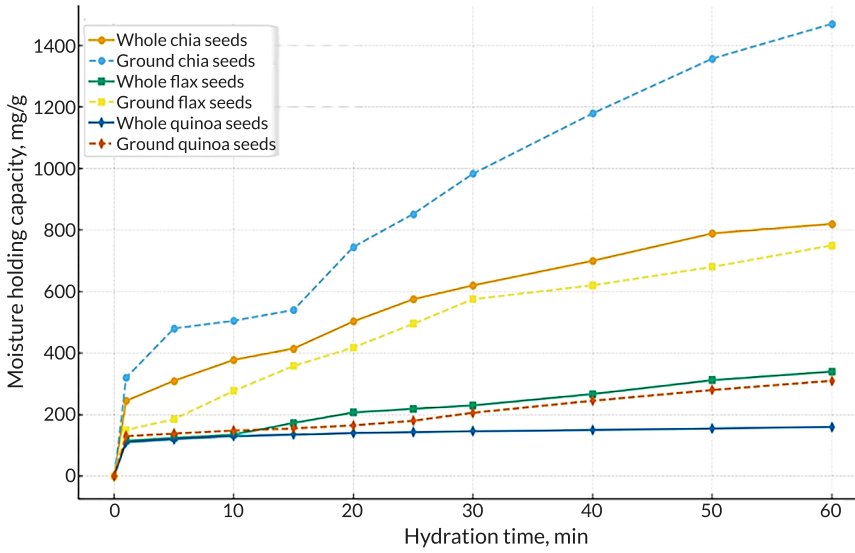

Fig. 1.1 Relationship between water-holding capacity and hydration time of whole and ground chia, quinoa, and flax seeds

Thus, chia, quinoa, and flax seeds should be used in ground form, with a particle size not exceeding 0.5 mm.

1.3.3 Refinement of formulations and technological operations for curd product manufacturing

Based on the results of previous studies, three formulations were developed and the technological parameters for producing new-generation curd products were refined. The formulations are presented in **Table 1.12**.

The manufacturing process of the curd product includes the following operations. Raw materials must comply with current regulatory documentation. Formulation components are weighed according to the developed recipes. Chia, quinoa, and flax seeds are ground to a particle size not exceeding 0.5 mm. Sea salt is sieved. To obtain the protein-fat base, low-fat cottage cheese is homogenized, soy-fat concentrate is added, and the mixture is thoroughly blended. During mixing, ground chia, quinoa, or flax seeds are incorporated into the protein-fat base. The resulting mixture is cooled and packaged.

Table 1.12 Formulations of the curd product

Raw material	Formulation		
	No. 1	No. 2	No. 3
Protein-fat base (fat content 8.0%), %	97.2	94.2	97.2
Chia seeds, %	2.0	–	–
Quinoa seeds, %	–	5.0	–
Flax seeds, %	–	–	2.0
Sea salt, %	0.8	0.8	0.8

1.4 Study of quality and safety indicators of the curd product

The nutritional value of the curd product is presented in **Table 1.13**.

Table 1.13 Nutritional value of the curd product

Indicator	Control	With chia	With quinoa	With flax
Protein (g/100 g dry matter)	17.34 ± 0.88	13.90 ± 0.70	13.68 ± 0.68	13.90 ± 0.70
Fat (g/100 g dry matter)	0.29 ± 0.02	10.03 ± 0.50	9.70 ± 0.49	10.25 ± 0.51
Carbohydrates (g/100 g dry matter)	6.68 ± 0.33	6.09 ± 0.31	8.41 ± 0.42	5.83 ± 0.29
Dietary fiber (g/100 g dry matter)	–	0.74 ± 0.04	0.42 ± 0.02	0.60 ± 0.03
Energy value (kcal/100 g)	98.72 ± 4.94	170.84 ± 8.54	175.66 ± 8.78	171.17 ± 8.56

The data in **Table 1.13** indicate that although the total protein content in the new curd products decreased by approximately 20%, the fat content increased nearly 30-fold due to the use of refined corn oil in the form of soy-fat concentrate.

The value of refined corn oil is determined by its favorable lipid profile, technological stability, and neutral organoleptic properties, making it particularly suitable for use in food products, including functional foods. A key indicator of the biological adequacy of the lipid component of food products is the ratio of omega-6 to omega-3 fatty acids. According to recommendations of the World Health Organization, the optimal ω-6/ω-3 ratio should range from 5 to 8, with the total intake of these polyunsaturated fatty acids providing approximately 1–2% of the daily energy intake [27].

An imbalance toward excessive omega-6 intake is considered one of the contributing factors to the development of chronic non-communicable diseases, including cardiovascular, oncological, inflammatory, and autoimmune disorders, as well as rheumatoid arthritis and asthma. Conversely, increased omega-3 consumption and a reduced ω-6/ω-3 ratio contribute to mitigating these adverse effects [28].

It should be noted that chia, quinoa, and flax seeds significantly influence the lipid profile of curd spreads, as these plant additives are valuable sources of polyunsaturated fatty acids, primarily α-linolenic acid (ω-3). Their high content contributes to enriching curd products with biologically active lipids, improving the ω-6/ω-3 ratio, and enhancing functional value. The presence of oleic acid (ω-9) positively affects fat system stability, while the low proportion of saturated fatty acids does not deteriorate the nutritional and dietary characteristics of the developed product.

Thus, the combination of corn oil with chia, quinoa, and flax seeds enables the formation of a balanced lipid composition in the curd product that meets modern requirements for functional foods, combining technological reliability with enhanced biological value.

The use of these plant ingredients expands the possibilities for developing domestic curd products for therapeutic and preventive purposes, integrating traditional technology with modern approaches to functional food design.

To determine microbiological safety, viable lactic acid bacteria, molds, and yeasts were analyzed two hours after production, along with the presence of coliform bacteria, *Salmonella spp*., and *Staphylococcus aureus*. Other samples were stored at 0–2°C for four days in accordance with DSTU 4503:2005 storage standards [29], with daily microbiological analysis.

The microbiological characteristics of the investigated curd product samples are summarized in **Tables 1.14–1.16**.

The results (**Tables 1.14–1.16**) demonstrate that the formulation components and technological parameters ensure microbiological purity of the curd product throughout the guaranteed storage period, comparable to classical curd paste.

It should be noted that the curd product containing flax seeds showed slightly higher counts of yeasts and molds compared to other samples. This may be explained by the presence of lignans (10.12–17.91 mg/g), mucilage substances, and phenolic compounds in flax seeds, which somewhat inhibit bacterial microflora but simultaneously promote moisture retention, creating favorable conditions for yeast and mold development [26].

Thus, the new type of curd product complies with industrial sterility standards.

Table 1.14 Microbiological parameters of curd product with chia seeds during storage

Parameter	Storage time, days					Standard
	0	1	2	3	4	
Lactic acid bacteria count in 1 g of product	10^7	10^7	10^7	10^7	10^6	$\geq 1 \cdot 10^6$
Coliform bacteria in 0.001 g of product	Not detected					Not allowed
Molds in 1 g of product, CFU	5 ± 0.25	5 ± 0.25	5 ± 0.23	6 ± 0.28	6 ± 0.31	≤50
Yeasts in 1 g of product, CFU	23 ± 1.01	23 ± 1.15	23 ± 1.14	23 ± 1.21	24 ± 1.23	≤100
Pathogenic micro-organisms, including *Salmonella*, in 25 g of product	Not detected					Not allowed
Staphylococcus aureus in 0.01 g of product	Not detected					Not allowed

Source: [29, 30]

Table 1.15 Microbiological parameters of curd product with quinoa seeds during storage

Parameter	Storage time, days					Standard
	0	1	2	3	4	
Lactic acid bacteria count in 1 g of product	10^7	10^7	10^7	10^7	10^6	$\geq 1 \cdot 10^6$
Coliform bacteria in 0.001 g of product	Not detected					Not allowed
Molds in 1 g of product, CFU	5 ± 0.21	5 ± 0.22	6 ± 0.19	6 ± 0.28	6 ± 0.27	≤50
Yeasts in 1 g of product, CFU	25 ± 1.11	25 ± 1.16	25 ± 1.09	26 ± 1.29	26 ± 1.22	≤100
Pathogenic micro-organisms, including *Salmonella*, in 25 g of product	Not detected					Not allowed
Staphylococcus aureus in 0.01 g of product	Not detected					Not allowed

Source: [29, 30]

Table 1.16 Microbiological parameters of curd product with flaxseed during storage

Parameter	Storage time, days					Standard
	0	1	2	3	4	
Lactic acid bacteria count in 1 g of product	10^7	10^7	10^7	10^7	10^6	$\geq 1 \cdot 10^6$
Coliform bacteria in 0.001 g of product	Not detected					Not allowed
Molds in 1 g of product, CFU	6 ± 0.12	6 ± 0.21	6 ± 0.18	7 ± 0.28	7 ± 0.29	≤50
Yeasts in 1 g of product, CFU	25 ± 1.01	25 ± 0.98	26 ± 0.99	26 ± 1.10	26 ± 1.12	≤100
Pathogenic micro-organisms, including *Salmonella*, in 25 g of product	Not detected					Not allowed
Staphylococcus aureus in 0.01 g of product	Not detected					Not allowed

Source: [29, 30]

1.5 Conclusions

The effectiveness of using the following ingredients in the technology of a new dairy-plant fermented curd product has been confirmed: soy-fat concentrate based on corn oil, flax seeds, chia seeds, quinoa seeds, and sea salt.

The studied nutritional indicators of the developed curd product demonstrated the advantages of the experimental formulation. Compared with the control, the new product is characterized by a higher content of beneficial fats and dietary fiber.

Comparison of the appearance and organoleptic properties of the developed curd product with those of the standardized sample showed the superiority of the experimental formulation across all studied indicators.

The developed curd product makes it possible to expand the range of functional dairy-plant fermented products.

Conflict of interest

The authors declare that there is no conflict of interest regarding this article or the published research results, including the financial aspects of conducting the study, obtaining and using its results, as well as any non-financial personal relationships.

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Authors' contributions

Larysa Bal-Prylypko: Conceptualization, Formal analysis, Supervision.
Halyna Tolok: Validation, Resources, Methodology, Formal analysis.
Semen Tolok: Investigation, Formal analysis.
Ihor Ustymenko: Writing – original draft preparation, Formal analysis.
Ivan Bal: Resources, Methodology.
Oleksandr Kanishchev: Resources, Methodology.

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CHAPTER 2

Monitoring of acarid mite populations in craft hard goat cheeses during ripening

Viktor Davydovych
Larysa Shevchenko
Olena Semenko
Nataliia Slobodyanyuk
Oksana Pylypchuk
Anastasiia Ivaniuta
Nataliia Holembovska
Alina Omelian

Abstract

The expansion of the assortment of artisanal hard cheeses ripened with the involvement of acarid mites on the food market necessitates the assessment of their safety. The use of mites contributes to the development of distinctive aroma and flavor profiles in mature and aged hard goat cheeses and requires the implementation of effective methods for regulating their population density. Four batches of cheeses produced from unpasteurized goat milk were manufactured at the Zhuravka Eco Farm (Kyiv region, Ukraine): Caciotta, Canestrato, Alpine, and Yogurt-type cheeses. Cheese heads at different levels of infestation with *Acarus siro* were selected for the study, and a methodology for monitoring mite density was developed. The most effective and practical method was the determination of mite density using a graduated grid and light microscopy, enabling the counting of mites at all developmental stages, including eggs. For unwashed cheeses, mite density is recommended to be determined in the surface powder accumulated on the rind and expressed per unit mass. For washed cheeses, mite numbers should be determined in rind scrapings and expressed per unit area. The number of *Acarus siro* mites in the "brown powder" accumulated on the surface of artisanal hard goat cheese heads ranged from 12.8 to 43.5 per 0.01 g. Insufficient control of mite population density during ripening resulted initially in superficial damage and subsequently in deep core deterioration, rendering the cheeses unsuitable for storage and consumption.

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Washing cheese heads with running water reduced mite density on the rind surface to 5.2–10.4/cm^2. Three months after washing, mite density reached 1.9–2.1/cm^2, while egg counts ranged from 9.9 to 11.7/cm^2 of rind. Treatment of hard goat cheese heads intended for sale with linseed oil resulted in complete elimination of *Acarus siro* mites and their eggs on the rind surface; however, it promoted secondary mold growth. Overall, the use of mites in the ripening of artisanal hard goat cheeses ensures the formation of distinctive sensory characteristics, which may serve as a criterion of product authenticity. Further research should focus on determining the aromatic composition of hard goat cheeses ripened with the participation of acarid mites.

Keywords

Collar pests, hard goat cheeses, craft production, method of counting mite density.

2.1 Introduction

One of the most common collar pests worldwide and in Ukraine is the acarid mite, in particular *Acarus siro* L. (Linnaeus, 1758), *genus Acarus* (Linnaeus, 1758), family *Acaridae* (Latreille, 1802). A feature of acarid mites is the ability of females to lay up to 800 eggs during their lifetime, and the development cycle lasts nine days. At the same time, the resistance of mites in the external environment is due to the ability of eggs to withstand a temperature of 0°C for several months. Mites belonging to the taxa *Acarina*: *Acaridae* are mainly characterized by small sizes and, when favorable conditions are created, which include optimal temperature and humidity, they multiply rapidly, which, without proper control of their population, leads to the appearance of various diseases in consumers and causes losses due to food losses.

Due to its physiological characteristics, *A. siro* is not picky about environmental conditions and is found in the temperate climate zone [1]. This species of mite often dominate other arthropod species in feed and food throughout the entire farm-to-fork chain. The spread of collar pests, such as *Acaridia* mites, to food products mainly occurs from the environment. They are found in warehouses, retail chains, ripening chambers for cheese and meat products, and in residential and non-residential premises. This mite often infests cereals, meat products, hard cheeses, various teas, dried fruits, and spices [2].

Damage caused by acarid mites to grain feeds often results in the loss of valuable seed material and feed suitability. Severe infestation of feeds and food products with mites causes unpleasant odors that consumers pay attention to.

Mites that breed in food products during storage can cause a variety of harm [3] in different ways:

– through direct contamination of various goods in the food chain;

– symbiotic relationships with microorganisms, including fungi and bacteria, which can increase the spoilage of a food product and lead to its loss of suitability for human consumption;

– production of allergens that may affect the health of the consumer.

As for allergens of mite origin in food products, they pose the greatest danger, since they induce IgE-mediated allergic reactions in humans, including anaphylactic manifestations. Such allergens are produced by four species of mites that infest food products. These include the following species: the American dust mite, *Dermatophagoides farinae* (Hughes) (*Acarina: Pyroglyphidae*), the scaly grain mite, *Suidasia sp. prob. pontifica* (Oudemans) (*Acarina: Suidasiidae*), the scaly mite of the genus *Saccharide*, *Thyreophagus entomophagus* (Portus & Gomez) (*Acarina: Acaridae*), and the mold mite, *Tyrophagus putrescentiae* (Schrank) (*Acarina: Acaridae*) [4].

These allergens can enter the human body not only directly – with food products infested with mites, but also indirectly through the respiratory system, by contact through the skin and mucous membranes, which provokes complications of allergic asthma, allergic dermatitis, intestinal and pulmonary allergies.

The scale of the spread of mites as grain pests and allergens is extremely large. Using data from the Czech Republic alone, where 514 grain silos were surveyed, 4 risk classes of grain mite allergy were identified, depending on the mite density: safe – 0 mites/g of grain; low – up to 1 mite/g of grain; high – 1–5 mites/g of grain; acute danger – more than 5 mites/g of grain.

At the same time, among the collar pests of grain crops, mites dominated, and their number reached 92%. Of these, 60% of mites belonged to species capable of producing allergens. The most common pests included *Acarus siro*, *A. faris*, *Tyrophagus putrescentiae* and *Lepidoglyphus destructor.* Accordingly, the obtained grain samples were classified according to allergic hazard as follows: safe – 37%, low – 53%, high – 6% and acute hazard – 4% [5].

As for the role of collar pests, in particular acarid mites, in the production of hard cheeses, two opposing aspects can be considered:

– harmful effects due to damage to cheeses during the ripening (storage) process;

– the beneficial role of mites in the ripening of hard cheeses is associated with the formation of exquisite sensory characteristics.

Regarding the harmful effects of mites, damage to cheeses can cause up to 25% weight loss in cheese wheels, especially in ripening chambers. Studies conducted in Spain have shown that damage by mites to Cabrales blue cheese, which ripens

in natural Asturian caves, deteriorates its presentation. This is facilitated by the conditions in natural caves, characterized by temperature fluctuations of 10–15°C and relative humidity over 90%. This temperature-humidity regime ensures the ripening of the cheese and, at the same time, corresponds to environmental parameters favorable for the reproduction and development of mites.

Mite damage is often a feature of long-matured cheeses, and the deterioration of Pecorino cheese has prompted the search for and use of cheese treatments, including ozonation during the ripening process [6]. For the fumigation of food products aimed at reducing mite infestation, liquid smoke, xanthan gum [7], sulphuryl fluoride, and methyl bromide are also employed, although the latter is subject to restrictions under the Montreal Protocol [8].

It is worth emphasizing the useful function of mites, which is inherent in the ripening process of refined elite hard cheeses. This is primarily due to the presence of opisthonotal glands in *Astigmata* mites, which are capable of producing a number of sensory compounds, in particular monoterpenes, cyclic and aliphatic volatile nitrogen-containing compounds, which give such cheeses their uniqueness. Moreover, these substances act as pheromones and fungicides. These include the German Milbenkäse cheese and the French Mimolette, Artisou, Laguiole, Salers and Cantal vieux cheeses. Most of these cheeses were characterized by taste and aroma compositions inherent in the mites *Tyrolichus casei* and *Acarus siro* L. It is believed that the mites *T. casei* produce neral, a compound that is responsible for the lemon flavor of cheeses and is concentrated in the rind, which gives them authenticity. When eating the cheese rind, consumers can feel this taste and enjoy it [9].

However, the literature reports only a few studies on the production, quality control, and safety of hard cheeses ripened with mite involvement. On the other hand, the expansion of the market for craft hard goat cheeses ripened with the participation of mites and the increasing popularity of gastronomic tourism require the development of criteria for the authenticity of such cheeses, as well as control of the number of mites dur ing their ripening.

Therefore, the purpose of this work was to develop a method for controlling the number of acarid mites during the ripening of craft hard cheeses made from unpasteurized goat milk, which are produced in Ukraine.

2.2 Damage to hard cheeses by mites during ripening or storage

The attractiveness of food and feed for acarid mites has long been known. In addition, these mites can feed on the mycelium of mold fungi, which also infect food

products, particularly cheeses. *Acarid* mites feed on a significant number of fungal species, including fungi used in the pharmaceutical industry and agriculture. Such fungi include many types of mold and yeast, in particular *Fusarium*, *Aspergillus*, *Candida*, *Hyphopichia*, *Penicillium*, *Rhizophus*, and *Trichophyton*. *Acarid* mites feed on spores and hyphae of fungi. This, in turn, ensures the spread of fungi by mites in the external environment. It has been proven that fungal spores are carried on the bodies of mites and are also excreted with their excrement.

Fungi, in turn, can cause cheese spoilage, creating both visible and invisible defects in the cheese wheels. The growth of fungi on the surface of the cheese rind causes the formation of metabolites that have unpleasant aroma, taste and texture, which is perceived by consumers as a deterioration in quality. Among the fungi that often contaminate hard cheeses and cause changes in their sensory properties, several genera are the most important, including *Penicillium*, *Aspergillus*, *Cladosporium*, *Geotrichum*, *Mucor* and *Trichoderma*. In addition to cheese spoilage, molds can pose a danger to cheeses by producing and accumulating mycotoxins. The risk of mycotoxin accumulation in cheeses increases significantly when cheeses are contaminated with fungi of the genera *Aspergillus* and *Penicillium*. The main causes of fungal contamination of hard cheeses during ripening are the contact of the wheels with contaminated air, as well as the ripening chamber equipment [10].

The nutritional value of fungi for acarid mites lies in the presence of cell walls containing chitin, as well as intracellular components such as trehalose. When consuming fungal hyphae, the mite digestive system produces a specific enzyme, trehalase, which is able to break down the contents of the hyphae, ensuring the digestion and assimilation of fungi. It has been proven that different species of acarid mites have individual preferences for the species composition of mold fungi [11].

Thus, hard cheeses are characterized by a double attraction for mites:

– due to mold fungi that grow on them and represent a source of nutrition;

– due to the consumption of hard cheese itself.

In the production of craft hard cheeses, the rind is usually not covered with protective films or paraffin and is an important factor that determines the attractiveness for consumers. The rind of hard cheeses forms a special taste, and its appearance is responsible for the authenticity of the product. A complex multi-species microbiome is created on the surface of the rind of hard cheeses, which spontaneously gets onto the surface of the cheese from both raw materials and the air of the ripening chambers, as well as equipment and tools used in the cheesemaking process. It is believed that the rind microbiome is significantly different from the core of the cheese wheels, due to differences in physicochemical characteristics at the surface and inside the wheels.

A feature of the environment on the surface of the cheese rind is a sufficient amount of oxygen, which causes the growth and reproduction of mold fungi, which belong to strict aerobes [12]. The formation of the rind microbiome of hard cheeses is significantly influenced by physicochemical characteristics, in particular pH, humidity and air temperature, as well as the humidity of the cheese wheels, cheesemaking technology, type, origin and blend of milk, and the quality and sanitary conditions of production.

Among the factors that determine the intensity of the spread, growth and reproduction of mold fungi on the surface of the rind of hard cheeses, moisture is in the first place. With a decrease in moisture in the environment, fungi belonging to the genus *Debaryomyces* grow better, as well as filamentous fungi of the genera *Aspergillus* and *Scopulariopsis*. They are the most common in cheeses with natural rind. They are characterized by resistance to the increase in the content of table salt, which is observed during the ripening of old hard cheeses [13].

Analysis of the abundance of molds and yeasts in craft hard cheeses made from unpasteurized goat milk showed their presence at all stages of ripening. Although some differences in their abundance and accumulation dynamics were noted between the ripening periods of Caciotta and Canestrato cheeses [14].

In young cheeses aged up to 3 months, intensive mite colonization of the wheels was not observed, which is probably due to incomplete colonization of the rind surface by mold fungi (**Fig. 2.1**).

Fig. 2.1 Wheel of young hard goat cheese Caciotta: 1 – the upper surface is covered with the mycelium of mold fungi

No less important factor is the relationship between different types of micro- and macrobiota in the chambers for ripening hard cheeses. As a rule, in such chambers, the penetration of mites onto the surface of the cheese wheels occurs naturally.

The mites fall off the cheese wheels located on the upper shelves of the ripening chamber and thus fall onto the surface of the cheese wheels located on the lower shelves. At the same time, during the ripening of craft hard goat cheeses, colonization of the wheels by mites is observed only if there is a sufficient amount of fungal mycelium on the upper, lower and side surfaces.

Accordingly, acarid mites begin to appear on the surface of only those cheese wheels that are covered with continuous mycelium of mold fungi (**Fig. 2.2**).

Fig. 2.2 Wheel of Alpine hard goat cheese: 1 – area of the rind cleared of mold fungi following colonization by *A. siro* mites

During this period, they begin to consume the mushrooms themselves, cleaning the cheese rind from their mycelium. Outwardly, this looks like the appearance of separate areas on the rind of cheese wheels, free from the mycelium of mold fungi. Such neutralization of mold fungi on the surface of the rind of hard cheeses has an important meaning, which is as follows:

– no need to specifically clean cheese wheels to remove mold fungi;

– the participation of mites in creating different intensities of amber color of the peel and giving the wheels an attractive presentation;

– partial destruction of the cheese rind, which affects moisture loss from the wheels and ensures the uniqueness of the texture, taste, and aroma during the ripening process.

As the ripening period of hard cheeses increases, acarid mites spread over the entire surface of the cheese rind and their presence can be identified by the appearance of a "greyish powder", which is a mixture of cheese residues, dead and live mites and their excrement (**Fig. 2.3**).

In this case, mites primarily eat the mycelium of fungi growing on the upper surface of the rind of hard cheese wheels, and then on the side and bottom surfaces. Later, when the mites have consumed all the mycelium of fungi that has grown on

the surface of the hard cheese wheel, they begin to consume the rind and the cheese itself, and a "brown powder" accumulates on its surface (**Fig. 2.4**). At this stage, fungal growth is practically not observed on the surface of the hard cheese wheels, and the entire wheel is covered with a continuous layer of "brown powder".

Fig. 2.3 Yoghurt cheese wheel with the upper surface completely populated by the *A. siro* mite:
1 – section of the lateral surface of the cheese wheel with remains of fungal mycelium;
2 – upper surface of the cheese wheel with "greyish powder"

Fig. 2.4 The wheel of Yoghurt cheese is completely inhabited by the mite *A. siro*, upper and lateral surfaces: 1 – "brown powder" on the surface of the wheel of cheese

The largest layer of such powder is usually observed on the upper surface of the cheese wheels, while on the lower and side surfaces a brown, powdery mass crumbles and is clearly visible on the racks and drainage mats where the cheese wheels are located in the ripening chambers.

If at this stage methods are not used to reduce the number of mites on the surface of cheese wheels, their density increases, and they continue to eat the cheese, creating more pronounced defects on its surface.

This manifests itself in the form of damage to the peel of varying degrees (**Fig. 2.5**), and subsequently to the core, which causes the rejection of such wheels with subsequent disposal.

Fig. 2.5 A slice of hard mature Canestrato cheese affected by a mite: 1 – superficial damage to the wheel of hard cheese by the mite *A. siro*

The greatest damage to the wheels of hard cheeses by acarid mites, to which *A. siro* belongs, is observed on their upper surface, which is associated with the cylindrical shape of the wheels and the peculiarities of their placement in the ripening chamber.

Particularly significant damage caused by mites in hard cheeses is associated with their local penetration through the rind. Such penetration of mites into hard cheeses is visually characterized by defects and holes on the surface of the wheel and on its lateral areas.

These holes can be placed randomly on the entire surface of the cheese wheels, their number depends on the density of the mite population, and the size can range from a few mm to 2–3 cm or more (**Fig. 2.6**). The shape of the holes made by mites

in the cheese rind can be round, oval, and most often irregular. Such holes can be of different depths:

– superficial (up to 2–5 mm);
– deep penetrating wheels.

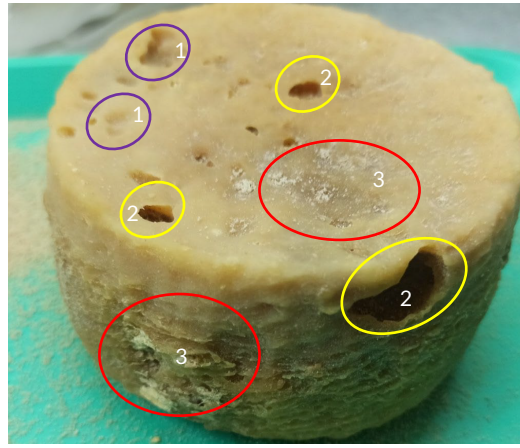

Fig. 2.6 Appearance of the wheel of hard goat Yoghurt cheese damaged by the *A. siro* mite: 1 – superficial; 2 – deep holes penetrating the core; 3 – deformation of the wheel surface

While the formation of surface holes and defects on the surface of the rind of hard cheese wheels is not harmful and even desirable, the penetration of mites into the core most often leads to spoilage of the wheels.

The formation of deep, penetrating holes in the rind of hard craft goat cheeses, in turn, causes deformation of its surface in various areas and ultimately leads to the loss of not only its presentation, but also its quality.

The surface of such a wheel of cheese resembles a "lunar landscape" and looks as if it has melted, which is explained by the varying intensity of mites eating the rind.

Sometimes there is local penetration of mites into the core of the cheese, and the wheel does not look damaged externally, but on the cut, it has characteristic traces of internal damage by the *A. siro* mite (**Fig. 2.7**).

In the early stages of mite penetration into the cheese core, changes in texture and color can be observed. Channels of various diameters made by mites in the cheese are visible, but the greyish powdery mass is not yet present (**Fig. 2.7**).

At a late stage of infestation of hard cheeses by mites, a crater-like area filled with greyish powder is clearly visible on the cut. This area is clearly demarcated from

the texture of the undamaged core, which is evident in the example of old-ripened goat cheese, Caciotta (**Fig. 2.7**).

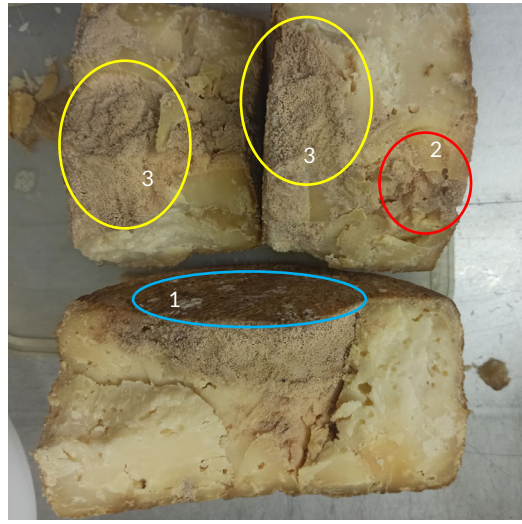

Fig. 2.7 Wheels of hard old-ripened goat cheese Caciotta in cross-section with varying degrees of damage to the core by the *A. siro* mite: 1 – internal damage to the core of hard cheese by the mite (the surface of the wheel has no visible defects on the outside); 2 – early stage of damage to the cheese core by the mite; 3 – late stage of damage to the cheese core by the mite

At the same time, in the core of such a wheel, a powdery mass of greyish color contains mites of various stages of development, their excrement and cheese residues.

When cut, such cheese has a specific odor, which is formed as a result of the vital activity of mites, as well as microbial processes accompanied by the breakdown of components of damaged cheese, excrement and dead mites. This odor often resembles ammonia and can vary in intensity depending on the degree of damage and the size of the hard cheese wheels.

Moreover, cheese wheels with such damage by core mites are not suitable for further storage or ripening and must be disposed of.

Many species of mites are common in Ukraine, which can infect food products and cheeses in particular. Analysis of the species affiliation of mites in individual regions showed that the dominant species of mites include *A. siro* and *T. putrescentiae.* Slightly less common are *Gl. burchanensis*, *Gl. destructor*, *Gl. domesticus*, *T. perniciosus*,

N. sokolovi, *N. rhizoglyphoides*, *Ch. arcuatus*, *G. fusca*, *Ct. plumiger*, *T. casei*, *Al. ovatus* and others. This explains the fact that the most common mites are *A. siro* and *T. putrescentiae*, which are isolated from food and feed produced and stored at various enterprises in Ukraine. As for other species of mites, *Gl. burchanensis*, *N. sokolovi* and *Gl. destructor* are less common as collar pests [2].

The entry of these and other types of mites into cheese ripening chambers and into the cheeses themselves is associated with their spread primarily in feed for ruminants, in particular goats. This is evidenced by analyses of feed samples taken from feeders and the floor of livestock premises. Such a spread of mites from livestock premises to the cheese manufacturing and ripening area is less typical for industrial production, since milk production and its processing are separated. However, for craft production, the spread of mites from feed and livestock premises to the cheese factory is quite possible, since farmers keep not only a herd of goats, sheep or other dairy animals, but also farm poultry. Mites can also be found in birdhouses where poultry are kept, fed, and watered. At the same time, milk production, its processing and sale of cheeses and other dairy products are concentrated on one farm, which significantly shortens the path of mites in the chain from "field to table".

Another factor in favor of the significant distribution of mites is their belonging to hygro- and thermophilic species, which allows them to tolerate adverse environmental conditions, survive, reproduce and spread. It has been established that such mites are able to form hypopuses in conditions of reduced temperature and relative humidity [2].

The prevalence of different mite species during cheese ripening has also been found in other studies, in particular, samples of Cabrales cheese ripened in caves contained representatives of *Acarus farris* (Oudemans) and *Tyrophagus neiswanderi* (Johnston and Bruce). This study notes that *A. farris* is not only the most common species, but also responsible for a significant part of the damage to Cabrales cheese [15]. Moreover, this mite can infect a wide range of other cheeses. It is believed that during cheese ripening, fungal growth occurs, which in turn stimulates an increase in the density of the mite population to a high level, which ultimately causes significant economic losses to producers. The prevalence of mite on Cabrales cheese causes a decrease in its marketability [16], which occurs due to the loss of weight of the cheese wheels, as well as an increase in the cost of workers involved in cleaning the wheels. The weight loss of cheese wheels can reach 2.5% and about 2% of additional costs are added to the cost of cheese due to the need to clean them from mites regularly. Before being sold to consumers, cheeses on which traces of mite activity are found require mandatory cleaning and control of remnants of collar pests. Such cheeses are recommended to be stored in refrigerators at a temperature of 2–4°C.

2.3 Beneficial effects of mites during the ripening of hard cheeses

In addition to the harmful effects of mites on cheeses, which are considered from the perspective of pests and the need to destroy them, there is a beneficial and desirable effect of certain species of mites during the ripening of hard cheeses. It has been found that mites in hard cheeses can give them specific and desirable sensory properties and thus make them unique and refined products. Cheeses that ripen with the participation of mites include quite well-known brands, in particular the French Mimolette and the German Milbenkäse [17].

Given that a large number of hard cheeses are produced worldwide and their range on the food market is constantly expanding, there is growing interest among scientists and producers in assessing their quality and safety, and in establishing criteria for authenticity. Some studies describe in considerable detail the production procedures for cheeses ripened with mite involvement [18].

The use of mites in cheese ripening has a history of over several hundred years. Two species of mites are used to make these cheeses, namely *Tyrolichus casei* (Oudemans) (*Tyrophagus casei* is a synonym of *Tyrolichus casei)*, which is specifically inoculated during the ripening of Würchwitzer Mühlenkäse cheese, and *Acarus siro* L., which is introduced into Mimolette cheese. The traditional Würchwitzer Mühlenkäse cheese, which has been certified as a "slow food" in Germany, has been produced since the Middle Ages, and its name comes from the region's ancient name.

This ancient technology of making cheeses ripened with mite involvement indicates that mites impart specific sensory properties that appeal to consumers who appreciate original cheeses. As a result of the analysis, it was concluded that ripening cheeses with these mites gives them a lemon flavor, which is formed due to the secretion of cheese mites [17].

The modern method of producing such cheeses is quite simple. Fresh sour milk cheese is made from cow's milk by fermentation with lactic acid bacteria. After that, caraway seeds and elderflowers are added to the cheese, elongated cylindrical loaves are formed and placed in a box with a colony of mites, where it ripens [19]. Such cheeses ripened with the participation of mites are unique and are in demand among gourmets.

Despite the fact that legislative documents are almost not developed for such products, the risks of mites and their waste products entering the body of consumers should be taken into account [18].

An analysis of the legislative framework for the control of mites in cheeses showed that these documents do not regulate the control of the species composition of mites during the ripening process of cheeses, but only determine their permissible number per unit of product.

The European Food Safety Authority (EFSA) regulations do not prohibit the use of live animals (including mites) in food for human consumption, but rather allow it [20]. The Codex Alimentarius [21] also does not set rules for the detection and control of mite numbers in cheese. However, legislation in various countries imposes restrictions on the import of cheeses with mites, in particular, the regulations of the United States of America [22]. Since 1940, the FDA has imposed a limit of 6 mites per square inch of cheese. Some samples of imported Mimolet cheese contained 4000 mites/square inch. In addition, the US Food and Drug Administration (FDA) provides for the use of regulatory action criteria for contaminants and extraneous materials to assess food adulteration. The criteria are divided into three categories: health hazards, sanitation indicators, and natural or unavoidable defects. Of particular importance is the category of human health hazards, which includes criteria for physical, chemical and microbiological hazards associated with contamination and the presence of extraneous contaminants. The category of consumer health hazards includes criteria for HACCP hazards (Hazard Analysis and Critical Control Points) and factors contributing to HACCP compliance. As for the sanitation indicators category, it includes criteria for visible undesirable contaminants, in particular, the penetration and infestation of commensal pests, which are associated with violations of sanitation requirements in food processing and storage facilities.

As for Brazilian legislation, Mimolet cheese, which is ripened with mites, is allowed to be imported, but its own production of a similar type of cheese is not provided for [23]. Current regulations only establish a maximum allowable number of mites in cheese, which should not exceed 25 dead mites per 225 g of cheese or 5 mite bodies on the surface of the cheese with an area of 2.5 cm^2 and a depth of up to 0.6 cm [23]. As for Canadian legislation, it is somewhat similar to Brazilian legislation and provides for the selection of three cheeses for analysis, while Brazilian legislation recommends taking only one sample [24].

The legislative framework of Ukraine, on the territory of which craft hard goat cheeses ripening with the participation of mites are produced, does not contain regulatory documents for such products, and the presence of mites on the surface of cheeses is assessed as a violation of storage conditions and damage by collar pests. Moreover, the "Methodological Guidelines on Compliance with Legislation on Food Safety and Certain Quality Indicators of Food Products at Primary Milk Production Facilities and/or Small-Scale Milk Processing Facilities" were developed and approved by the Minister of Agrarian Policy and Food of Ukraine on 1 May 2025. This guideline includes a separate paragraph 6 "Measures to control cheese mites". This paragraph states that if the cheese ageing room is infected with mites, all infected cheese, packaging and other materials must be removed from the room. After that,

it is recommended to clean the room thoroughly, especially the ceiling, walls, floor, racks, shelf supports, and the shelves themselves. To eliminate mites, vacuum packaging of cheese, washing it with hydrogen peroxide, regular washing and cleaning of cheese, as well as treatment of the legs of racks with "diatomaceous earth" are recommended. All workers who have come into contact with infected cheese are allowed to perform any procedures with other uninfected cheeses only after thorough washing and changing into clean work clothes.

Regarding the use of effective means of neutralizing mites, the use of diatomaceous earth is considered the most promising not only in the food industry, but also in the production of feed. However, the search for effective acaricides is not limited to this. Testing of inert materials, in particular zeolite and kaolin for neutralizing the cheese mite *Tyrophagus putrescentiae* (Schrank) (*Astigmata*: *Acaridae*) on wheat showed their acaricidal effect. When these preparations were applied to wheat grains at doses of 100, 500 and 1000 ppm, the death of *T. putrescentiae* was detected after 3 and 7 days. This study shows the superiority of zeolite over kaolin in terms of acaricidal activity. This is evidenced by the death of 100% of adult mites found in wheat grains treated with zeolite at a concentration of 1000 ppm, already 3 days after treatment. Treatment of wheat grain with kaolin at all doses was less effective in inactivating *T. putrescentiae* [25].

Similar information on cheese mites is provided by the Codex Alimentarius, which recommends the use of various methods of eliminating mites, including chemical fumigation, biological preparations, anaerobic or refrigerated food storage conditions [26].

Thus, most Ukrainian legislative documents and international requirements treat the presence of mites in cheeses as pests rather than as a component of the biome. This is primarily due to sanitary and hygienic requirements for the production, storage, and sale of hard cheeses. Although the absence of viable or dead mites on the surface of hard cheeses does not guarantee the absence of their waste products. Therefore, another important fact must be taken into account when analyzing cheeses ripening with the participation of mites is hidden infestation by mites. Since cheese can be processed or stored in conditions unsuitable for the reproduction of mites, their number can be reduced to a minimum or not detected at all. However, their waste products can be found on the surface of cheese, which can potentially cause allergies in a certain category of consumers [15].

In addition, the identification and determination of the mite population density on the surface of hard cheeses, which are characterized by a long ripening period and do not allow the wheels to be covered with protective films or paraffin, may be important to producers of these unique products themselves. Detection of mites on the surface of the rind of hard cheeses will allow for effective regulation of their density, timely prevention of damage to the wheels and loss of marketability, as well

as informing consumers about possible health risks when consuming products made with the participation of mites.

2.4 Development of a method for controlling the number of mites during the ripening of craft hard goat cheeses

2.4.1 Identification of mites in hard goat cheeses

Isolation and identification of mites was carried out in the laboratory of the Department of Animal and Food Hygiene named after A. K. Skorokhodko of the National University of Life Resources and Environmental Sciences of Ukraine and in the laboratory of the Department of Zoology of Uzhhorod National University, Ukraine.

To determine the density of mite infestation of hard craft goat cheeses, three names were used: Yoghurt with a ripening period of 6 months and 18 months, Canestrato with a ripening period of 18 months, and Caciotta with a ripening period of 20 months. The cheeses were made according to the recipe described earlier by us [14, 27] under the conditions of the Eco farm "Zhuravka" in the Kyiv region (Ukraine) from the milk of one herd of Anglo-Nubian goats.

The following characteristics were used to identify and differentiate *Acarus siro: A. siro* has a slit on the posterior part of its body between the 2nd and 3rd pairs of legs. Male *A. siro* has tarsal and anal suckers on its body, as well as a distinct hook-like extension on the segments of the first pair of legs. Females have a claw at the end of each leg [28].

Cheeses were used to develop a method for controlling mite density during ripening and storage. During cheese production, mites were introduced to the surface of the cheese rind naturally from the ripening chamber; no special inoculation was performed.

The colonization of the surface of hard goat cheeses with mites depended on the intensity of mold growth. In the future, the ripening process of cheese wheels took place with the participation of mites, and the wheels of experimental cheese samples were not subjected to treatment.

In the example of Yoghurt cheese, superficial damage to the rind of the wheels is clearly visible, especially after washing with running water. This indicates the presence of a viable colony of *A. siro* mites (**Fig. 2.8**).

Ripening of craft hard goat cheeses with the participation of cheese mites *A. siro* provided a gradual increase in the intensity of the rind color from light yellow to dark amber. The increase in the intensity of the rind color of hard cheeses directly depended on their age.

Fig. 2.8 Surface of washed wheels of Yoghurt cheese aged 4 months, ripened with the participation of the *A. siro* mite after washing with water: 1 – superficial damage to the rind by the mite

Thus, wheels of Yoghurt cheese aged 4 months had a light amber rind with traces of superficial damage to the rind by mites on the surface (**Fig. 2.8**), and in cheeses aged 6 months the rind color noticeably changed to amber (**Fig. 2.9**). In the cross section, wheels of such cheese had a clear demarcation of the texture from the rind.

Fig. 2.9 Wheel of washed Yoghurt cheese aged 6 months, ripening with the participation of the *A. siro* mite after washing with water on the cut: 1 – texture of hard cheese on the cut with a clear demarcation of the rind

2.4.2 Methodology for determining the density of mites on the surface of cheese wheels

To count mites, the 15 × 15 grid of 1 mm × 1 mm squares, 96° pharmacopoeial ethyl alcohol, microscope slides and coverslips, a scalpel for taking scrapings from the surface of cheese rinds, and an OHAUS NV212 (OHAUS CORPORATION, USA) scale with an accuracy of 0.01 g and a microscope MBS 9 (Ukraine), which allows for observation in both artificial and natural lighting were used.

To sample the powder on the surface of the cheese wheels or perform scrapings, the cheese wheel was conditionally divided into 4 sectors, which were further divided into 3 sub-sectors and 3 samples of "brown powder" were taken from each, which was located on the upper, lower and lateral surfaces of the wheel (**Fig. 2.10**). The sampling scheme from the upper and lower surfaces of the wheel is identical. From the lateral surface of the cheese wheels, 3 samples were also taken diagonally, covering the lower, middle, and upper thirds of the wheel's height. Thus, in this case, 12 samples can be taken from each surface of the wheels of hard cylindrical cheeses for an objective assessment of the density of acarid mites.

To analyze mites, a 0.01 g sample of "brown powder" was taken, placed on a glass slide, and then a drop of 96° ethyl alcohol was applied to immobilize the mites and facilitate counting, and covered with a coverslip.

To analyze the density of mites in washed cheeses, or cheeses washed and treated with linseed oil, samples were taken from the surface of the rind by scraping with a scalpel with an area of 1 cm × 1 cm. In this case, the mass of the sample ranged from 0.01 to 0.03 g.

Some regulatory documents state that a 6 mm-deep slice of cheese rind should be selected [23], but in this study, this method was ineffective because cheese contains significant amounts of fat and protein, which interfere with microscopy. In addition, it was not detected *A. siro* mites in cheese slices, they were located on its surface, which is due to their need for oxygen for respiration.

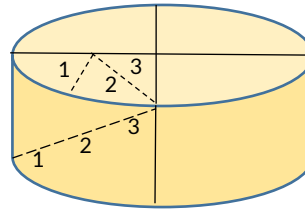

Fig. 2.10 Sampling scheme for analysis of mite density in cylindrical cheeses

The layout of the graduation grid, object and cover glass is shown in **Fig. 2.11**.

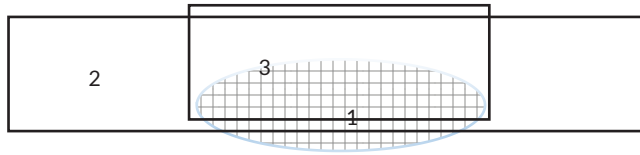

Fig. 2.11 Scheme of microscopy of cheese samples for counting the number of mites: 1 – calibration grid; 2 – slide; 3 – cover glass

For mite counting, the use of a microscope slide is not essential; however, in its absence, the mesh must be cleaned of any remaining cheese crumbs and mites, and degreased, after each count.

The slide with the cheese sample was placed on a calibration grid (**Fig. 2.12**) and microscopy was performed at a magnification of ×8.

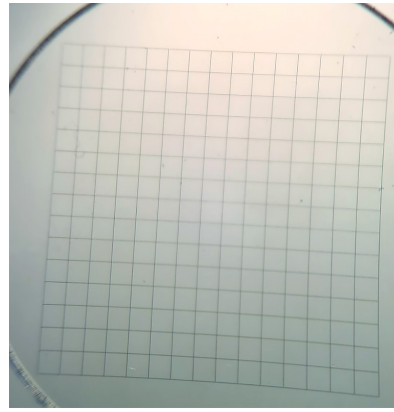

Fig. 2.12 View of the calibration grid for counting mites in the field of view of the microscope (magnification ×8)

The method of counting immobilized mites is as follows: in the first row, all mites were counted from left to right, as well as all those located in the center, on the upper and lower lines of the horizontal rows of the grid. In the second and all subsequent rows, all mites located in the center and on the lower line of the row were counted (**Fig. 2.13**). The counting direction for odd lines was from left to right, for even lines – from right to left.

1.	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2.														
3.														
4.														
5.														
6.														
7.														
8.														
9.														
10.														
11.														
12.														
13.														
14.														
15.														

Fig. 2.13 Scheme of counting mites in horizontal rows of the calibration grid

The microscope lens clearly shows the lines of the graduation grid at different magnifications, on which immobilized mites at different developmental stages are placed. They are located in the center and on the lines of the grid cells. Around the mites, a powdery mass is visible, taken from the surface of the cheese wheels (**Fig. 2.14**).

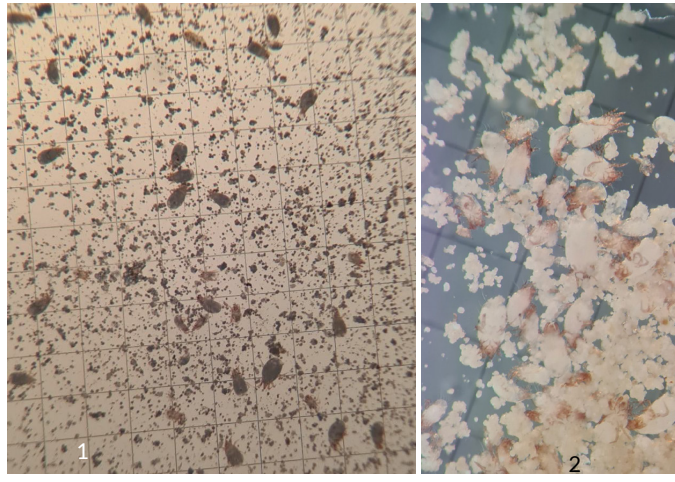

Fig. 2.14 Fragment of a calibration grid with immobilized mites *A. siro* under a microscope: 1 – sample 0.01 g; 2 – sample 0.02 g

For ease of counting, it is possible to select a mass of "brown powder" from cheese wheels that will allow an objective assessment of the density of mites using a calibration grid. In this study, a mass of 0.01 g was the most acceptable, while a weight of 0.02 g or more caused the accumulation of mites and cheese grains in the field of view of the calibration grid, which made it difficult to count them.

Counting the density of *A. siro* mites in the field of view of a microscope using a graduated grid showed that in 0.01 g of "brown powder" taken from the upper surface of the wheel of Yoghurt cheese, there were from 34 to 59 individuals of different stages of development. On the lower surface of the cheese wheel, their number was somewhat lower, ranging from 16 to 31 mites. The lowest density of mites found in the "brown powder" from the lateral surface of the cheese wheel was from 6 to 21 individuals (**Table 2.1**).

Table 2.1 *A. siro* mite density in the "brown powder" on the surface of craft hard goat cheese Yoghurt

Sample No.	Pieces/225 grid squares (in 0.01 g of "brown powder")		
	Upper surface of the wheel	Underside of the wheel	Lateral surface of the wheel
1	59	25	17
2	34	22	13
3	46	16	21
4	38	17	15
5	55	29	7
6	35	23	12
7	39	31	10
8	46	18	19
9	44	17	14
10	40	29	6
11	36	19	9
12	50	24	11
x ± SD	43.5 ± 8.0^{a}	22.5 ± 5.2^{b}	12.8 ± 4.6^{c}

Note: different superscript letters indicate a significant difference between the values in a row of the table using the Tukey's test ($p \leq 0.05$)

Accordingly, the density of *A. siro* mites on the underside of the cheese wheels was 48.3% lower, and on the lateral surface it was 3.4 times lower compared with the upper surface. This pattern is due to the shape and placement of the wheels in the ripening chambers. From the sides and lower surfaces of the cheese wheels, part

of the mites fall together with the powder onto the drainage mat, whereas from the upper surface this does not happen.

Further ripening of craft hard goat cheeses in a ripening chamber, as well as preparation of finished wheels for sale, involves washing with running water followed by drying on drainage mats.

As the study results demonstrated, on the surface of the freshly washed wheels of Yoghurt cheese, the density of *A. siro* mites sharply decreased compared with the corresponding values recorded prior to washing (**Tables 2.1, 2.2**). However, it should be noted that washing the wheels of Yoghurt cheese under laboratory conditions did not allow achieving the standard mite density on their surface. Density of *A. siro* mites in 1 cm^2 scrapings from the upper and lower surfaces of the wheel of Yoghurt cheese did not differ significantly, while on the lateral surface, it was lower by 1.88–2.00 times, respectively. No eggs of *A. siro* mites were detected in scrapings from the surfaces of the cheese wheels.

To monitor the rate of mite colonization on cheese surfaces, Caciotta cheese wheels were used; after washing, the wheels were dried on a draining mat and left in the maturation chamber. Studies have shown a slightly lower density of *A. siro* mites on the surface of aged Caciotta cheese.

Table 2.2 *A. siro* mite density in scraping from the surface of craft hard goat cheese Yoghurt immediately after washing with running water

Sample No.	Pieces/225 grid squares		
	Upper surface of the wheel	Underside of the wheel	Lateral surface of the wheel
1	6	8	4
2	15	12	3
3	7	10	6
4	11	11	7
5	10	13	4
6	14	11	5
7	12	14	6
8	8	12	7
9	6	7	4
10	9	7	8
11	11	7	9
12	12	12	11
x ± SD	9.8 ± 3.1^a	10.4 ± 2.3^a	5.2 ± 1.6^b

Note: a scraping from 1 cm^2 of cheese was taken for analysis, different superscript letters indicate a significant difference between the indicators in the table row using the Tukey's test ($p \leq 0.05$)

Washing of Caciotta cheese wheels followed by drying on drainage mats and storage in a refrigerated state for 3 months in a craft factory environment showed low densities of *A. siro* mites in crust scrapings (**Table 2.3**).

This cheese was characterized by the absence of a significant difference in the density of mites on the upper, lower, and lateral surfaces of the wheels.

It should be noted that the analysis of the number of *A. siro* mites in scrapings from different parts of the cheese wheel indicates a slightly lower density than in Yoghurt cheese. Probably, *A. siro* mites inhabit hard goat cheeses in different ways, which depends on their recipe and manufacturing technology.

Thus, on the upper surface of the wheels of Caciotta cheese, the number of mites per 1 cm^2 of scrapings ranged from 0 to 5 individuals, on the lower surface – from 0 to 4, and on the side – from 0 to 5 in 225 squares of the calibration grid.

No statistically significant difference was observed between the mean densities of *A. siro* mites on different areas of the cheese wheels. Moreover, when considering the regulatory limits for mite density in cheeses, this value did not exceed the permissible level established by Brazilian legislation, which specifies a maximum of five mites on a 2.5 cm^2 surface of cheese to a depth of 0.6 cm [23].

Table 2.3 *A. siro* mite density in scrapings from the surface of craft hard goat cheese Caciotta 3 months after washing with running water

Sample No.	Mite density, pieces/225 grid squares		
	Upper surface of the wheel	Underside of the wheel	Lateral surface of the wheel
1	3	1	4
2	2	3	0
3	5	4	4
4	0	1	2
5	2	3	3
6	0	2	2
7	1	3	0
8	3	0	5
9	4	4	0
10	1	2	1
11	4	0	2
12	0	0	0
x ± SD	2.1 ± 1.7^a	1.9 ± 1.5^a	1.9 ± 1.8^a

Note: a scraping from 1 cm^2 of cheese was taken for analysis, different superscript letters indicate a significant difference between the indicators in the table row using the Tukey's test ($p \leq 0.05$)

Analysis of scrapings of Caciotta cheese after washing with running water and storing for 3 months in a refrigerated state showed the presence of eggs of the *A. siro* mite in scrapings from the upper, lower, and lateral surfaces of the cheese wheels (**Fig. 2.15**).

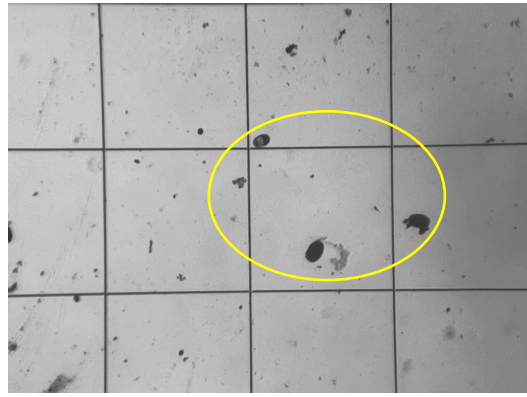

Fig. 2.15 Fragment of a calibration grid under a microscope with mite eggs in scrapings of Caciotta cheese after washing with running water and storing for 3 months in a refrigerated state

Under the microscope, eggs of *A. siro* mites have a clear, oval shape, allowing them to be differentiated from the cheese mass grains and other components in the field of view of the grading grid. The grains, which contain the remains of cheese and mite waste products, are characterized by irregular shapes, different sizes, and colors. This is clearly visible in the microscope's field of view and allows them to be separated from mite eggs. At the same time, this method does not allow to differentiate live mites from dead ones, since under the influence of 96° ethyl alcohol they have an immobilized appearance and do not differ from each other.

Despite the fact that no regulatory documents worldwide contain restrictions on the density of mite eggs in cheeses, this study conducted such an analysis because it is a necessary condition for predicting the risks of mite reproduction and spread in cheeses.

The results of the study showed that the density of mite eggs did not differ significantly in samples taken from the upper, lower and lateral surfaces of Caciotta cheese wheels. It ranged from 5 to 16 on the upper surface, from 4 to 17 on the lower surface and from 7 to 17 on the lateral surface in 225 squares of the calibration grid (**Table 2.4**).

Table 2.4 Mite egg density in scrapings from the surface of craft hard goat cheese Caciotta 3 months after washing with running water

Sample No.	Pieces/225 grid squares		
	Upper surface of the wheel	Underside of the wheel	Lateral surface of the wheel
1	7	4	14
2	12	11	10
3	11	10	15
4	10	16	12
5	9	12	17
6	5	6	9
7	14	17	10
8	10	10	15
9	16	8	8
10	12	9	11
11	7	11	7
12	9	5	13
x ± SD	10.2 ± 3.1^a	9.9 ± 3.4^a	11.7 ± 3.1^a

Note: a scraping from 1 cm^2 of cheese was taken for analysis, different superscript letters indicate a significant difference between the indicators in the table row using the Tukey's test ($p \leq 0.05$)

Thus, it can be concluded that washing the cheese wheels with running water not only fails to completely remove mites from the surface but also leaves viable individuals capable of reproduction.

Calculation of the mite population density on the surface of hard cheese indicates a fairly significant error in the results, which ranges from 26.5% to 34.3%. However, the developed method may satisfy the purpose of most studies, since it is quite difficult to achieve high accuracy in calculating the mite density. This is primarily due to the movement of mites on the surface of cheese wheels, and their density can vary both spatially and temporally.

Among methods for isolating and recording mite density in feed and food products, the method developed by M. Solomon [29] is still used. This method involves determining the density of mites in bulk samples of feed or food products, followed by counting them in sectors of a Petri dish. The results are expressed in units of volume, which for hard cheeses cannot be an objective indicator of mite infestation, since their highest density is on the surface, i.e. in the scraping or "brown powder" on the surface of the wheels. This does not reflect the density of infestation of the entire wheel of hard cheese and does not take into account the uniformity of the distribution

of mites, which is due to the peculiarities of its texture, in particular the high content of moisture, protein and fat, which interfere with the microscopy process.

To analyze the density of mites on the surface of hard cheeses, it is more appropriate to use two methods:

– counting the number of mites in the "brown powder" that is on the surface of the wheels. In this case, the calculation can be made per unit mass;

– counting the density of mites on the surface of washed or washed and oiled cheeses. In this case, it is advisable to scrape from the surface of the rind and calculate per unit area.

The developed method is simpler, more convenient, and takes less time. In addition, this method is non-destructive, which allows samples to be taken from the surface of cheese wheels and then placed in chambers for further ripening or sale to consumers.

The reagents, equipment and materials used in this method do not require preparation, are quite cheap and do not require a special recipe or permit for purchase. This also indicates the possibility of its use in any cheese factory. The use of a calibration grid manufactured industrially eliminates the error in the calculations due to the unified size of the cells and their number, which allows for an increase in the accuracy of the calculation. At the same time, the calibration grid is fully within the microscope's field of view, reducing errors in counting mites within cells or rows. Furthermore, the developed method allows for the enumeration of not only mites but also their eggs, thereby enabling the prospective prediction of their distribution on food products. This method also does not depend on the correctness and accuracy of the researcher in manually making a graduated card for a Petri dish with division into sectors, which involves the method developed by M. Solomon [29].

2.4.3 Methods for reducing mite density during ripening and storage of craft hard goat cheeses

Among the methods proposed to reduce the density of mites on the surface of cheeses during their ripening process, the use of chemicals is proposed, in particular, fumigation with methyl bromide (currently banned by the Montreal Protocol), as well as the use of registered organophosphorus insecticides. At the same time, it is also prohibited for neutralizing the *A. farris* mite that damages Cabrales cheese. This is due to the insecticide's toxicity, primarily to consumers.

There is an ongoing discussion in the literature about the effectiveness of using acaricidal agents based on insect growth regulators, inert dust, plant-based

preparations, and individual representatives of the pyrethroid insecticide group. However, such agents must meet the requirements of the "One Health" concept, i.e., take into account the impact on human, animal, and environmental health [30]. This can be achieved by meeting the following criteria:

– high acaricidal effectiveness;
– harmlessness to human health;
– no effect on the organoleptic characteristics of cheese;
– low cost, ease of use, dosage and storage;
– harmlessness to the environment and non-target organisms.

The recommended means of preventing the spread of mites on food products, in particular cheeses, primarily include natural components. To neutralize mites that damage food products, such as cured ham, coating with vegetable oils or hot lard is used. It is also considered quite effective to use essential oils that contain biologically active compounds – monoterpenes. Monoterpenes of essential oils of plants showed high acaricidal activity and did not have a significant effect on the sensory properties of food products. They are also considered safe for consumer health.

Another strategy to control mite populations in cheeses is to regulate the relative humidity in the ripening chamber. Results showed that *A. farris* population density decreased at relative humidity levels below 70%. However, this was ineffective at controlling mite populations on Cabrales cheese [15]. The researchers concluded that the cheese's high water content provided the mites with suitable conditions for reproduction, and that the humidity in the ripening chamber was not significant.

Comprehensive studies on the effectiveness of coating with fatty acid, monoterpene (eucalyptol) at low temperatures to control the population density of *A. farris* in cheeses indicate that their population density on cheeses ripened at temperatures of 2, 4 and 6°C was significantly reduced. Thus, at a temperature of 2°C, 10 mites/cm^2 were detected, at a temperature of 4°C – 11.4 mites/cm^2 and at 6°C – 14.7 mites/cm^2 against 174.33 mites/cm^2 in the control. For all temperature regimes, these values were also significantly lower than the initial values (10 versus 21.3 mites/cm^2 at 2°C, 11.4 versus 22.4 mites/cm^2 at 4°C, and 14.7 versus 26.5 mites/cm^2 at 6°C) [16].

Analysis of Ras cheese samples showed that it was infested simultaneously with three mite species: *A. siro* and *A. farris* (family *Acaridae*), as well as *Carpoglyphus lactis* (family *Carpoglyphidae*). In this study, the authors showed that the dominant mite species infesting Ras cheese was *A. siro*, with an abundance of up to 82% of the other mite species. Another study also found that cheeses were infested with *A. siro* [31].

Also, the main means for neutralizing mites in cheese were proposed to use essential oils of cloves, citrus crops, thyme and rosemary. Their effectiveness was proven in the treatment of Ras cheese from *A. siro* mites. The acaricidal effect

depended on the type and concentration of the essential oil used to treat the cheese. Among the essential oils, clove oil was the most effective. LD50 (oral dose for mites, providing 50% mortality of mites) for a 0.1% concentration of clove oil provided 95% mortality of mites. A higher concentration of clove oil guaranteed 100% death of mites on cheese. Slightly lower efficiency in terms of LD50 for mites was found in thyme and rosemary oils. They were effective at a concentration of 1.0%, which provided mortality of mites at the level of 75% and 55%, respectively. With increasing concentrations of both oils, the mortality of mites increased. It is believed that the essential oils of the above-mentioned plants can be an effective means of protecting Ras cheese from mite infestation during ripening, storage and sale. The authors also emphasize that the essential oils of plants can exhibit a bactericidal effect, which can have an effect on microorganisms necessary for the life of mites. At the same time, such treatments can affect certain properties of the cheese and the health of consumers. Treatment of cheese with essential oils does not exclude a change in its sensory characteristics, which can in some way affect consumer preferences. This study confirmed that essential oils of plant origin change the organoleptic properties of Ras cheese: the taste and smell of this cheese improved when treated with citrus or thyme essential oils [28].

To control mite density in hard cheeses, our study used two strategies (**Fig. 2.16**):

– reducing the density of mites in cheeses subject to further ripening by washing with running water;

– decontamination of mites in cheeses to be sold by washing with running water and treating with linseed oil.

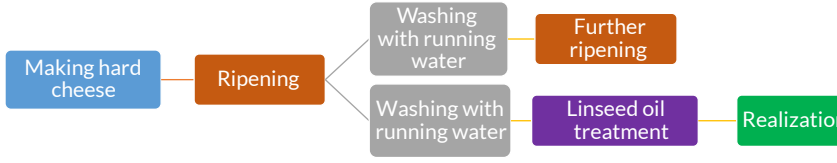

Fig. 2.16 Scheme of processing of craft goat hard cheeses to control the density of *A. siro* mites

The use of linseed oil for the processing of craft hard goat cheeses, Yoghurt and Canestrato, showed its high acaricidal efficiency. This is confirmed by the analysis of scrapings from different surfaces of their wheels (**Table 2.5**).

No mites or their eggs were detected on the surface of Yoghurt cheese 2 months after washing and treatment with linseed oil, as well as Canestrato cheese 3 months after washing and treatment with linseed oil.

Table 2.5 Density of *A. siro* mites in scrapings from the top, bottom, and side surfaces of craft hard goat cheeses after washing with running water and treating with linseed oil

Sample No.	Yoghurt cheese (2 months after treatment)		Canestrato cheese (3 months after treatment)	
	Presence of mites	Presence of mold fungi	Presence of mites	Presence of mold fungi
1	0	+	0	+
2	0	–	0	–
3	0	–	0	+
4	0	+	0	–
5	0	–	0	+
6	0	+	0	+
7	0	–	0	–
8	0	+	0	–
9	0	–	0	+
10	0	–	0	–
11	0	+	0	–
12	0	+	0	+
x ± SD	0 ± 0^a	White mold	0 ± 0^a	Blue mold

Note: a scraping of 1 cm^2 of cheese was taken for analysis; "+" – presence of mold growth, "–" – absence of mold growth

Considering that linseed oil is a valuable food product and does not affect the organoleptic characteristics of hard cheeses, it can be considered a natural acaricidal agent for storing craft cheeses intended for sale to consumers.

At the same time, the presence of white and blue mold was noted on their surface, which indirectly confirms the absence of mycoid mites *A. siro* on the wheels of cheese (**Fig. 2.17**).

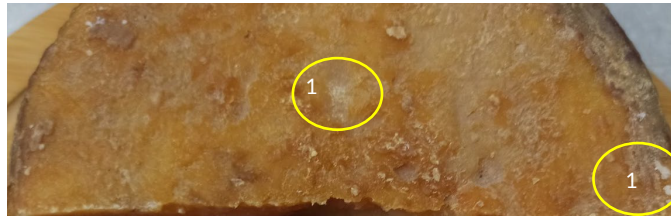

Fig. 2.17 Yoghurt cheese washed with running water and treated with linseed oil: 1 – traces of white mold

The growth of mold fungi on the surface of craft hard goat cheeses can recur due to their contamination with spores during the ripening process. The absence of mycoid mites or a reduction in their population density on the surface of cheeses to a critically low level can promote the germination of spores on the surface of cheese wheels. This phenomenon is also observed in craft hard goat cheeses used for this study. Many researchers believe that the growth of fungi on the surface of hard cheeses can have certain benefits, since it provides the creation of more complex aroma and taste compositions of cheeses, thereby forming original sensory properties.

At the same time, an important criterion for assessing the safety of hard cheeses on which mold fungi grow is the production of mycotoxins. Their formation depends on the ripening period of the wheels and requires strict control of the temperature and humidity regime in the ripening or storage chambers. Therefore, characteristics such as the level of relative humidity and the air temperature of the ripening chambers are key for the growth of fungi. No less important are the indicators of the physicochemical composition of the cheeses themselves, in particular the pH value, the content of table salt and the activity of water. These parameters can also be used to regulate the intensity of the growth of mold fungi on the surface of cheeses [32].

If fungal mycelium appears on the surface of the cheese wheels, they can be mechanically removed. This technique was used to clean the craft hard goat cheeses in this study. A similar practice is used for Brazilian craft cheese, which is ripened in the cheese factory premises to remove white filamentous fungi.

It is believed that the presence of fungal growth on the surface of cheeses does not always indicate the production of mycotoxins by them. For example, PR-toxin, as one of the strongest, synthesized by certain strains of the fungus *P. roqueforti*, does not accumulate in cheeses during ripening in quantities dangerous to consumers. This is due to the lack of optimal conditions for the synthesis of this mycotoxin, since such conditions are not provided in cheeses [33].

Among the most common fungi isolated from craft cheeses, the main ones are representatives of the genera *Aspergillus* and *Penicillium*. For the synthesis of mycotoxins, a fairly high water activity is required, which should be 0.96–0.99, as well as a low pH level of about 5.0. These conditions are able to ensure the activation of genes that determine toxinogenesis. In addition, the intensity of mycotoxin formation depends on the ambient temperature, which should be 25–30°C [34]. These parameters differ significantly from the parameters provided in the ripening chamber of hard cheeses. In this study, hard craft goat cheeses were used, which ripen at a temperature of 8–11°C, which is significantly below the optimal regime.

It should be noted that the ripening period of hard cheeses also affects their mycobiome. In this study, the Caciotta cheese used was characterized by the presence of

mold fungi only at the age of 1 month, and at the age of 12 and 24 months, they were not detected, while in Canestrato cheese, the growth of mold fungi was recorded at the age of 10 days, 3 and 12 months [14]. As for the Alpine and Yoghurt cheeses, which were also used in this study, they had their own characteristics of mold fungus growth. In Alpine cheese, the peak of mold fungi occurred at the 6th month of ripening, but they were also detected at the age of 12 months. In Yoghurt cheese, mold fungi were isolated only at 6 months of age, and during the ripening periods of 7 days and 18 months, their colonies were not detected [27]. Thus, it can be assumed that the growth of mold fungi on hard craft goat cheeses, even those made from the same raw milk, differs significantly. This may depend on the recipe and the manufacturing technology.

Fresh cheeses are thought to contain more aflatoxins than mature or aged cheeses, due to the presence of proteins capable of binding them. The ripening process of cheeses is also associated with certain chemical processes that cause changes in the mycotoxin molecules, which may reduce the risk to the health of consumers [35]. Although cheese is a nutrient medium for many species of filamentous fungi, they do not always produce toxins or produce them in quantities that do not pose a health risk. Among the mycotoxins found in cheeses, the most common are penicillic acid, mycophenolic acid (MPA), cyclopiazonic acid (CPA), roquefortine C, sterigmatocystin (STC), aflatoxin, PR-toxin and citrinin, which are not able to be stored for a long time. The low content of mycotoxins on the one hand and the small portion of consumption of old-ripened cheeses also on the other hand reduce the risk of mycotoxin poisoning of the body [36]. Thus, it can be concluded that the appearance of mold on the rind of cheese wheels does not always indicate their danger to humans.

The ineffective fight against molds that grow on cheese rinds ultimately led producers to diversify their cheese production. This led to increased investment in the production of cheeses that have a surface-ripened rind with molds, which contributed to the creation of much more complex aromatic compositions and flavors compared to traditional craft cheeses. Such cheeses have reached a new stage of development in the food market due to their increased value.

The problem of contamination of hard cheeses with fungal spores has not yet been solved [37]. However, this situation has served to achieve success in the production of cheeses with a swollen crust, which occurs only with the participation of autochthonous mycoflora on the surface of the cheese crust. In this case, harmful fungi have been used to produce unique cheeses with unique sensory characteristics. Although, to assess the risk, it is necessary to conduct a study not only of the mycobiota of hard cheeses, but also to clarify their relationship with the mites that spread to such cheeses. It is known that certain preferences regarding the species composition of fungi also characterize acarid mites. For example, *A. siro* mites did

not consume the mycelium of black-colored fungi, while they preferred the mycelium of white- and blue-colored fungi on the rind of craft hard goat cheeses. In this study, significant attention was not paid to the species composition of mold fungi growing on the rind of hard goat cheeses during ripening, although in production conditions, there is a practical method that allows for mechanical cleaning of the mycelium of black-colored fungi. In this case, the cleaned areas of the rind of hard cheeses are colonized by other species of mold fungi.

As for the danger of mycotoxins, which are capable of producing mold fungi in cheeses, the vast majority of studies have not confirmed the presence of mycotoxins, and the assumption that they are dangerous to the health of the consumer, which is based only on the detection of mold fungi on their surface, is questionable [38].

Thus, undesirable and sometimes dangerous representatives of mycobiota, as well as acarobiota on hard cheeses [39, 40] have shown their important role in the creation of new dairy products – hard cheeses with original sensory properties. Therefore, it is worth noting the need to review the hazard criteria for craft hard goat cheeses, which are made in compliance with good manufacturing practices. In this regard, it is also important to distinguish the following concepts in regulatory documents:

– contamination of hard cheeses with mites as collar pests;

– inoculation of mites as a component of the microbiome of hard cheeses.

The results obtained in this study can be the basis for developing effective methods for controlling the number of mites during the ripening process of hard cheeses and assessing their participation in the creation of original, authentic products that meet the criteria of taste pleasure.

2.5 Conclusion

It was established that for the correct ripening of craft hard cheeses from goat milk with the participation of acarid mites, it is necessary to control their population density. An effective method for counting the number of mites for unwashed and washed cheese wheels has been developed. The essence of the method for determining the number of mites for unwashed cheeses is to establish the optimal weight of powder from the surface of the wheels, and for washed cheese wheels – to scrape an area of 1 cm^2 from the surface of their rind, then immobilize the mites using 96° ethyl alcohol and count them on a graduated grid in the field of view of a microscope. This method also provides for the possibility of counting mite eggs, which allows predicting the intensity of their reproduction and spread.

Craft hard goat cheeses, Caciotta, Canestrato, Alpine and Yoghurt are colonized by *A. siro* mites starting from 3–4 months in ripening chambers. The density of *A. siro* mites in the "brown powder" on the surface of cheese wheels reaches 12.8–43.5 in 0.01 g. The uncontrolled process of reproduction and spread of mites on the surface of hard goat cheeses during ripening causes their surface damage, which is characterized by deformation of the rind and the appearance of surface holes. Subsequently, mites penetrate into the core of the cheese wheels with subsequent damage and unusability.

To control the density of acarid mites on the surface of the wheels of craft hard cheeses intended for further ripening, they are washed with running water. This ensures a decrease in the density of mites on the surface of the cheese rind 3 months after washing to the level of 1.9–2.1/cm^2, and their eggs to 9.9–11.7/cm^2 of rind.

For wheels of hard cheeses intended for sale, it is possible to treat with cold-pressed linseed oil, which ensures complete neutralization of *A. siro* mites and their eggs. During storage of wheels of hard cheeses treated with linseed oil, regrowth of mold fungi on the surface of the rind was observed.

For unwashed and untreated hard goat cheeses that ripen with the involvement of acarid mites, the density of mites on the surface of the cheese wheels is not regulated. For hard goat cheeses that ripen with the involvement of acarid mites and are subsequently washed with running water, a density of mites not exceeding 2.5/cm^2 and an egg density not exceeding 15 per cm^2 may be regarded as acceptable.

For hard cheeses washed with running water and treated with oils intended for sale, the presence of mites on the surface of the wheels is not permitted.

The use of *A. siro* mites for ripening craft hard goat cheeses may be one of the criteria for their authenticity and involves, in the future, instrumental research of their sensory characteristics and assessment of consumer demand.

Conflict of interest

The authors declare that there is no conflict of interest regarding this article or the published research results, including the financial aspects of conducting the study, obtaining and using its results, as well as any non-financial personal relationships.

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The manuscript has no associated data.

Use of artificial intelligence statement

The authors confirm that no artificial intelligence technologies were used in the preparation of this work.

Authors' contributions

Viktor Davydovych: Conceptualization, Methodology, Empirical research design, Data analysis, Writing – original draft.

Larysa Shevchenko: Conceptualization, Methodology, Empirical research design, Data analysis, Writing – original draft.

Olena Semenko: Validation, Language editing, Interpretation of results, Writing – review and editing.

Nataliia Slobodyanyuk: Data curation, Literature review, Formal analysis, Visualization, Writing – review and editing.

Oksana Pylypchuk: Validation, Formal analysis, Interpretation of results, Writing – review and editing.

Anastasiia Ivaniuta: Data curation, Literature review, Formal analysis, Visualization, Writing – review and editing.

Nataliia Holembovska: Data curation, Literature review, Formal analysis, Visualization, writing – review and editing.

Alina Omelian: Validation, Formal analysis, Interpretation of results, Writing – review and editing.

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CHAPTER 3

The effect of protective composition on the quality of sweet cherry during storage

Olesia Priss
Iryna Ivanova
Marina Serdyuk
Tetiana Tymoshchuk
Olga Pyurko
Sergii Basanets

Abstract

The effect of an exogenous protective composition based on lactic and acetic acids on the preservation of sweet cherry fruits during refrigerated storage was studied. The physiological and biochemical mechanisms of action of this composition were established. The object of the study was sweet cherry fruits (*Prunus avium* L.) of nine pomological varieties: Rubinova Rannia, Valerii Chkalov, Kazka, Talisman, Dilema, Melitopolska chorna, Karina, Regina, Krupnoplidna. Samples were selected in the phase of consumer ripeness, taking into account varietal uniformity, degree of ripeness and fruit diameter. Before storage, the fruits were precooled: by intensive air method, hydrocooling in water and a combined method, which involved hydrocooling in aqueous solutions of organic acids with subsequent cooling with cold air. The cooled fruits were stored in a cold room at a temperature of 1.5 ± 0.5°C and a relative humidity of 93 ± 1%. The fruits were stored in cooling conditions for 40 days. The results suggest that the formation of the integral loss index is determined primarily by the method of pre-cooling the fruits, while varietal characteristics modify, but do not determine the nature of the post-harvest preservation of the fruits. The maximum yield of standard fruits was recorded in the experimental version of combined cooling with the use of an exogenous protective composition. It was found that cooling with a protective composition based on organic acids contributed to the reduction of the oxidative load in the tissues of sweet cherry fruits after 40 days of storage, which is confirmed by a lower level of malondialdehyde and a more balanced response of antioxidant enzymes.

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Taking into account the experimental data obtained, the practical significance of the proposed technology acquires a clearly expressed economic value. Under the conditions of selling 1 ton of chilled cherries, the revenue is 23 thousand UAH, while the value of the additional net profit obtained for the implementation of the proposed technology increases by almost 12,92 thousand UAH per 1 ton of product. The socio-economic effect of increasing the yield of standard products when stored using a combined method is 2918,2 UAH per 1 ton of fruit.

Keywords

Sweet cherry, cooling, storage, lactic acid, acetic acid, oxidative stress, malondialdehyde, antioxidant enzymes, microbiological diseases, physiological disorders, standard products, post-harvest losses.

3.1 Introduction

Sweet cherry (*Prunus avium* L.) belongs to the most valuable stone fruit crops, characterized by high consumer attractiveness in the fresh state, a limited storage life, and increased sensitivity to transportation and storage parameters. The global market of fresh sweet cherry exhibits stable demand, whereas international trade is concentrated in specific production regions and exhibits a pronounced seasonal pattern. This determines increased requirements for the stability of quality attributes and the predictability of product preservation within the cold chain system. Marketability of sweet cherry is determined not only by product mass but also by compliance with established standards regarding external appearance and textural characteristics [1]. Therefore, under cold chain transporting conditions, even a minor increase in the share of fruit exhibiting defects can significantly reduce the commercial value of the respective lot.

The scale of post-harvest losses also has a broader systemic dimension. According to estimates by the Food and Agriculture Organization of the United Nations, the global share of food loss at stages from harvest to retail amounts to approximately 13.3%, with fruit and vegetable products traditionally being among the most vulnerable categories due to high moisture content, intensive metabolism, and sensitivity to mechanical damage and microbial contamination [2]. Therefore, the reduction of post-harvest losses of fruits is considered one of the most effective approaches to increasing food system efficiency and reducing the resource burden on agri-food systems [3]. For sweet cherry, this issue is particularly acute: the crop belongs to highly perishable fruits, in which quality is determined by a delicate balance between physiological senescence, tissue water status, and the suppression of microflora growth.

The biological characteristics of sweet cherry significantly limit storage duration in the absence of specialized post-harvest regulatory technologies. Sweet cherry fruit possess a relatively delicate skin, high water content, and active post-harvest metabolism, which create preconditions for rapid senescence and texture loss. Simultaneously, during cold storage and transportation, the risk of microbiological diseases increases, representing one of the principal causes of marketability loss and reduction of the marketing period [4].

Contemporary studies on sweet cherry storage indicate that quality degradation in the post-harvest period is multifactorial and caused by: (i) physiological senescence accompanied by disruption of tissue structural integrity; (ii) development of oxidative stress, accumulation of malondialdehyde, and imbalance of antioxidant enzymes; (iii) microbiological contamination of the fruit surface followed by the development of microbial diseases under favorable conditions [5]. In addition, even under optimal temperature regimes, mechanical impacts during harvesting and sorting may cause localized tissue injuries, which intensify quality losses and increase fruit susceptibility to infection by pathogenic microorganisms [6]. In response to these challenges, post-harvest technologies in recent years have increasingly shifted from traditional approaches toward exogenous stability regulators with an improved safety profile. They combine antimicrobial effects, minimal risk of undesirable changes in sensory properties of product, and reduced technological and regulatory barriers. Recent research highlights growing interest in eco-friendly, safe postharvest solutions that effectively regulate physiological and biochemical processes in fruits, curb microbial diseases, preserve sensory qualities, and align with modern sustainability standards [7].

For sweet cherry, several approaches of exogenous regulation have proven their effectiveness, such as application of physical treatments and modified atmospheres, or natural bioactive compounds exhibiting antioxidant and antimicrobial properties [8]. However, the efficiency of such approaches is variable and depends on cultivar, maturity stage, pre-harvest conditions, and storage parameters, thereby necessitating the search for simple, reproducible, and technologically compatible solutions.

In this context, organic acids are considered a promising group of exogenous regulators, as they are capable of influencing the microbiological stability of the fruit surface through pH reduction, disruption of proton homeostasis in microbial cells, and inhibition of key metabolic processes. Recent review studies emphasize that organic acids are widely used as preservatives and acidity regulators and may demonstrate effectiveness against a broad spectrum of bacteria, yeasts, and fungi, being active under different temperature regimes. From the standpoint of post-harvest fruit

physiology, acid treatments also have the potential to indirectly affect the intensity of oxidative processes in tissues by reducing microbially induced damage and stabilizing surface barriers, which collectively may contribute to texture preservation and a reduction in the proportion of defects during storage [9].

Therefore, the relevance of studies devoted to investigating the effect of an exogenous protective composition based on lactic acid and acetic acid on the quality parameters of sweet cherry fruit during storage is determined by a number of interrelated factors: (i) the high commercial sensitivity of the crop to even moderate decreases in marketability; (ii) the dominant role of microbiological diseases and physiological senescence in the formation of post-harvest losses; (iii) the need for technologically simple, reproducible, and clean-label compatible solutions; and (iv) the necessity to move from fragmented quality indicators toward an integrated assessment of lot quality, in which the key criterion is the proportion of standard products after prolonged storage.

This conceptual framework – linking the regulation of microbiological stability and oxidative processes with the ultimate indicator of commercial quality – constitutes the scientific basis and determines the practical significance of the present study.

3.2 Theoretical foundations for the application of organic acids in post-harvest treatment

Lactic and acetic acids are low-molecular organic acids, the antimicrobial activity of which is due to a combination of physicochemical and biochemical mechanisms of action on the cells of microorganisms. Compared with other bactericidal and bacteriostatic substances, the mechanism of action of organic acids does not involve a direct effect on the process of protein denaturation and does not change the cellular structures of microorganisms. Organic acids in an undissociated form penetrate semipermeable membranes and dissociate. Protons formed as a result of dissociation reduce intracellular pH, and thereby disrupt the acid-base and energy balance of microbial cells. This causes acid stress. Under such conditions, the microbial cell is forced to spend a large amount of energy not on growth and reproduction, but on overcoming the effects of acid stress. This leads to rapid depletion of energy reserves and slowing down its growth [10].

Anions formed as a result of dissociation accumulate inside the cell and interfere with metabolic processes. They block the work of enzymes, disrupt logistical chains, the synthesis of proteins, DNA and other important metabolites. It should be noted that the mechanism of destructive action of anions is not universal and differs for

different types of acids. Thus, anions of acetic acid – acetates – mainly disrupt the energy chains of the microbial cell, which are connected with acetyl – CoA. In turn, anions of lactic acid – lactates – unbalance the redox system of microbial cells and inhibit the processes of their respiration [11].

When choosing organic acids for the formation of protective antimicrobial compositions for the treatment of fruit products, it is also important to take into account their physicochemical properties, as well as the conditions of use, in particular concentrations, temperature indicators, quantitative and species composition of microflora. Particular attention should be paid to the ratio of dissociated and undissociated forms of acids, which is determined by the pH indicator. The lower the pH value, the more undissociated forms in the solution and the more pronounced the antimicrobial effect [9].

The intensity of these mechanisms is determined by the physicochemical characteristics of the acids themselves and by environmental conditions. The effectiveness of lactic and acetic acids depends on their acid dissociation constant (pK_a), concentration, temperature parameters, and the composition of the surface microbiota of the fruit. Under post-harvest treatment conditions, particular importance is linked to the ratio between dissociated and undissociated forms, since the latter determines membrane permeability and the expression of bacteriostatic or fungistatic effects [12].

However, the mechanism of action of organic acids during post-harvest processing is not limited to the level of the microbial cell, but extends to the microenvironment of the fruit surface and its apoplastic space. The result of processing with protective compositions containing organic acids is an increase in the acidity of the cuticular layer and the surface layer of the epidermis, which inhibits contamination and further development of pathogenic microflora on the surface of storage objects [13]. The increase in the level of active acidity on the surface of fruit products affects not only the activity of microorganisms, but also inhibits the activity of enzymes. As a result, the rate of tissue destruction during storage decreases [14, 15]. Thus, post-harvest treatment with organic acids forms an acid barrier on the surface of storage objects, which reduces the risk of their microbiological spoilage during further storage.

Reducing the level of microbial contamination and the intensity of the development of microorganisms during storage has a positive effect on endogenous metabolic processes that occur during the storage of fruit products. The process of storing fruit raw materials is accompanied by the development of oxidative stress, the consequence of which is the accumulation of reactive oxygen species (ROS). First of all, these are hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and hydroxyl radical (•OH). Intensive formation of reactive oxygen species induces peroxidation of cell membrane lipids, loss of ionic balance and accelerated tissue destruction [16].

Damage to plant tissues due to microbial contamination activates defense mechanisms, depletes them more quickly, and stimulates the development of oxidative stress [17]. On the other hand, reducing the level of contamination and the intensity of the development of pathogenic microflora when treating the surface of fruits with solutions of organic acids does not deplete the antioxidant system, reduces the level of formation of reactive oxygen species and inhibits the development of oxidative stress [18].

Therefore, the mechanism of action of organic acids in the composition of protective compositions for post-harvest processing of fruit products is characterized by both an external antimicrobial effect and indirect regulation of oxidative processes within tissues. This contributes to the extension of the shelf life of fruits with minimal loss of quality.

3.3 Conceptual framework and experimental modeling of the process

The scientific hypothesis of the study is based on the assumption that the application of an exogenous protective composition based on lactic and acetic acids, in complex with a combined cooling method, forms a controlled acidic barrier at the "surface – apoplast" interface of sweet cherry fruit. Such modification of the microenvironment is expected to reduce microbial load, limit pathogen penetration through micro-injuries, stabilize cellular membranes, and indirectly regulate the intensity of oxidative processes in the tissues. The implementation of these mechanisms is expected to reduce share of non-marketable fruit and increase the yield of standard products during prolonged cold storage.

Within the framework of this hypothesis, several working assumptions were formulated. It was proposed that the effectiveness of the acid composition may depend on cultivar-specific characteristics and the morphological structure of the fruit skin; that combined cooling would promote a more uniform temperature gradient within the tissues; that a reduction in microbial load would correlate with decreased lipid peroxidation levels; and that changes in apoplastic pH could serve as an early indicator of the depth of exogenous treatment effects and the stability of the cell wall structure.

The aim of the study was to provide experimental substantiation of the effect of an exogenous protective composition based on lactic and acetic acids on the storability of sweet cherry fruit under cold storage conditions and to elucidate the physiological and biochemical mechanisms underlying its action.

To achieve this aim, the following objectives were addressed:

– determination of the magnitude of microbiological and physiological losses during 40 days of storage;

– assessment of changes in natural weight loss and in the proportion of standard (marketable) products;

– calculation of the integral loss index and the share of standard products;

– investigation of the activity of antioxidant enzymes in fruit tissues;

– quantification of the intensity of lipid peroxidation based on malondialdehyde (MDA) content;

– analysis of the dynamics of apoplastic pH as an indicator of the acid–base status of the extracellular space;

– establishment of the relationships among microbiological, physiological, and biochemical quality indicators.

The object of the study was sweet cherry (*Prunus avium* L.) fruit of several varieties during the post-harvest period under cold storage conditions.

The subject of the study comprised changes in quality indicators, microbiological stability, and the redox status of fruit tissues under the influence of an exogenous protective composition based on organic acids within a combined cooling system.

At the experimental stage, the objects of analysis were fruits of nine sweet cherry cultivars: Rubinova Rannia, Valerii Chkalov, Kazka, Talisman, Dilema, Melitopolska chorna, Karina, Regina, and Krupnoplidna. Samples were collected at the stage of consumer maturity, taking into account cultivar uniformity, degree of ripeness, and fruit size (diameter).

After harvesting, the fruits were sorted to remove mechanically damaged and defective specimens and were bulk-packed into plastic crates (600 × 400 × 116 mm) with a net weight of 10 kg. Subsequent operations were carried out according to the developed experimental design, which included three pre-cooling treatments:

1. Control 1 (C1) – intensive air cooling. Cooling was performed using a forced stream of cold air at a velocity of 3.0 m/s with an air exchange rate of 90 volumes per hour. The chamber temperature was maintained at 0 ± 1°C with a relative humidity of 90 ± 1%.

2. Control 2 (C2) – hydrocooling. Cooling was carried out in a stationary pallet hydrocooler MAS-HC-2000-PAL-ST (capacity 2 t/h) using water at a temperature of 1.0 ± 0.5°C.

3. Experimental treatment (R) – combined cooling with an exogenous protective composition. The combined method consisted of two sequential stages. The first stage involved cooling in water at 1.0 ± 0.5°C supplemented with lactic acid (2.16%) and acetic acid (1.71%). The cooling duration was 10 ± 2 min, until the fruit core temperature reached 4 ± 1°C. The concentrations of organic acids were determined in preliminary studies as optimal for maintaining product quality and safety [19]. The second stage consisted of subsequent air cooling in a chamber with intensive air

circulation at a velocity of 3.0 m/s (90 volumes per hour) for 30 ± 2 min, until the fruit core temperature decreased to 2 ± 0.5°C. The operating temperature in the chamber was maintained at 0 ± 1°C with a relative humidity of 93 ± 1%. The total duration of the combined process was 40 ± 2 min.

After cooling, the fruits were placed in storage at a temperature of 1.5 ± 0.5°C and a relative humidity of 93 ± 1% in modernized cold chambers KH-48 equipped with an Eliwell EWDR 902 temperature control system and Eliwell EWHS 31 relative humidity sensors. The storage chambers were fitted with a battery-type cooling system. Temperature within the internal tissues was monitored using a digital thermometer TM-902CP with a type K thermocouple (measurement range −50 to +1300°C; resolution 0.1°C within −50 to 200°C). Each experimental treatment was performed in five replicates; one standard 10 kg crate constituted a single replicate.

To determine the effect of the exogenous protective composition on sweet cherry fruit quality during storage, a comprehensive evaluation of indicators was conducted, characterizing both the commercial suitability of the product and the biochemical mechanisms underlying its stability.

At the applied level, following parameters were analyzed: losses due to microbiological diseases, the proportion of fruit affected by physiological disorders, the magnitude of natural weight loss, and the share of standard (marketable) products after 40 days. Additionally, an integral index of reduction in commercial lot yield was calculated as a generalized indicator of product storability under cold storage conditions. Assessments were carried out in accordance with current regulatory methodologies, with changes monitored throughout the entire storage period.

Fruit exhibiting signs of microbiological infection and physiological disorders were recorded by complete inspection of each lot after every storage stage. The proportion of defective fruit was expressed as a percentage of the total number or mass of the sample according to the following formula

$$X = \frac{m_d}{m_0} \cdot 10, \tag{3.1}$$

where m_d – the mass of defective fruit, kg; m_0 – the initial mass of the specimen, kg.

Natural weight loss was determined by a gravimetric method through periodic weighing of crates containing fruit using analytical scales with an accuracy of 0.01 kg. Weight loss (%) was calculated as the ratio of the difference between the initial and current mass to the initial sample mass.

The proportion of standard (marketable) products was established in accordance with the requirements of current regulatory standards for the commercial quality of sweet cherry fruit.

The integral index of reduction in commercial lot yield was calculated as the total share of losses resulting from microbiological diseases, physiological disorders, and natural weight loss, allowing a comprehensive assessment of treatment effectiveness over 40 days of storage.

To provide a scientific substantiation of the mechanism of action of organic acids, biochemical indicators reflecting the state of oxidative homeostasis in fruit tissues were included in the evaluation system. In particular, the activity of key antioxidant enzymes – superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) – involved in ROS detoxification and regulation of cellular redox status was determined.

For enzymatic analyses, pulp samples (without stones) weighing 5–10 g were collected and immediately homogenized in chilled phosphate buffer (0.05–0.1 M, pH 7.0–7.8) at a ratio of 1:5 (w/v). The homogenate was centrifuged at 10,000–15,000 rpm for 15–20 min at 4°C. The supernatant was used to determine enzyme activity. All procedures were performed at low temperatures. Results were calculated on fresh weight (FW).

POD activity was determined by titration of residual hydrogen peroxide following pyrocatechol oxidation and expressed as μmol $H_2O_2 \cdot g^{-1} \cdot min^{-1}$.

CAT activity was measured based on the rate of hydrogen peroxide (H_2O_2) decomposition by recording the decrease in absorbance at 240 nm. Activity was calculated from the change in H_2O_2 concentration over time and expressed as μmol $H_2O_2 \cdot g^{-1} \cdot min^{-1}$.

APX activity was determined by monitoring the rate of ascorbate (AA) oxidation in the presence of hydrogen peroxide, recording the decrease in absorbance of the reaction mixture at 290 nm. Activity was calculated using the molar extinction coefficient for ascorbate ($\varepsilon = 2.8\ mM^{-1} \cdot cm^{-1}$) and expressed as μmol $AA \cdot g^{-1} \cdot min^{-1}$.

SOD activity was determined based on its ability to inhibit the auto-oxidation of adrenaline in an alkaline medium, following the methodology in [20] with adaptation of the plant material preparation stage for analysis. For the assay, 0.5 g of finely ground plant tissue was homogenized in 5 mL of phosphate buffer (pH 10.65), using a mortar and pestle with added cooling medium (on ice) to prevent enzymatic degradation. The resulting homogenate was transferred to centrifuge tubes, after which 0.3 mL of chloroform and 0.6 mL of ethanol were added to precipitate impurities. The mixture was centrifuged at 8,000 rpm for 20 min. The supernatant was used for subsequent analysis, and the change in optical density was measured spectrophotometrically at 347 nm. SOD activity was expressed in arbitrary units as the percentage inhibition of adrenaline auto-oxidation relative to the control.

The intensity of lipid peroxidation was assessed by measuring MDA content using the thiobarbituric acid (TBA) reaction. After heating the reaction mixture and centrifugation, optical density was recorded at 532 nm with correction for non-specific absorption at 600 nm. MDA concentration was calculated using the molar extinction coefficient and expressed in nmol · g^{-1} of FW.

Additionally, apoplastic pH dynamics were monitored to assess changes in the acid-base status of the extracellular space and to determine the presence of a pH gradient at the "surface – apoplast" interface. Apoplastic pH was measured using a vacuum infiltration method with an isotonic solution, followed by centrifugation of the tissue to obtain apoplastic fluid. The pH of the extract was measured with a calibrated pH meter equipped with a microelectrode at 20 ± 1°C. These values were used as an indicator of the acid-base status of the extracellular space in fruit tissues.

All determinations were performed in five biological replicates. Statistical processing of experimental data was carried out using Microsoft Excel 365 software (Microsoft Corp., USA). The results were presented as the mean and standard deviation. To assess the influence of the cooling method, variety and their interaction, a two-factor analysis of variance (two-factor ANOVA) was used. Differences were considered statistically significant at $p \leq 0.05$. To characterize the share of the influence of individual factors, the η^2 indicator was additionally determined.

3.4 Effect of exogenous protective composition on the formation of sweet cherry fruit losses during storage

The effectiveness of postharvest treatments for sweet cherry fruits should be evaluated not only by individual quality indicators but primarily through a system of quantitative loss characteristics that develop throughout the storage period. The structure of total losses is multi-component and includes natural weight loss, determined by the intensity of transpiration and respiration; losses due to microbiological damage associated with the development of phytopathogenic microflora; and losses caused by physiological disorders resulting from disruption of water balance, membrane stability, and enzymatic equilibrium in the tissue. The combined effect of these factors ultimately determines the yield of standard (marketable) products and the economic efficiency of the storage technology.

Stone fruits, including sweet cherries, are characterized by high respiration rates, thin cuticles, and increased sensitivity to mechanical damage, creating conditions for rapid development of both dehydration and microbiological contamination. Over a 40-day storage period, even minor changes in microbial load or tissue

water-retention capacity can lead to nonlinear increases in the proportion of non-marketable fruit. Therefore, quantifying each loss component is essential for justifying the applied treatment methods and comparing their effectiveness across different cultivars.

Given the multifactorial nature of postharvest degradation processes, there is a need for a unified criterion that integrates diverse types of losses into a single quantitative measure. In this study, the chosen criterion is the integral loss index, which reflects the total reduction in commercial yield as a result of the three main components.

The integral index of commercial yield reduction of the lot (I_{Σ}, %) was calculated as the sum of three loss components formed during fruit storage: losses due to microbiological damage, losses from physiological disorders, and natural weight loss.

The proportion of losses due to microbiological damage (L_{mic}, %) was determined as follows: after sorting the lot, fruits showing signs of microbial spoilage were selected. The loss fraction was calculated based on mass

$$L_{mic} = \frac{m_{mic}}{m_0} \cdot 10, \tag{3.2}$$

where m_{mic} – the mass of fruit with microbiological damages, kg; m_0 – the initial mass of fruit lot, kg.

The proportion of losses due to physiological disorders (L_{phys}, %) was determined as follows: fruits exhibiting pronounced physiological defects that rendered the fruit non-marketable without signs of rot (such as softening, wilting, pedicel darkening, or subcutaneous spotting) were selected. The loss fraction was calculated using the formula

$$L_{phys} = \frac{m_{phys}}{m_0} \cdot 100, \tag{3.3}$$

where m_{phys} – the mass of mass of fruits with physiological disorders, kg.

It should be noted that each fruit was counted in only one defect category (based on the dominant symptom) to avoid double counting.

Natural weight loss (L_{nw}, %) was calculated as the relative decrease in lot mass due to transpiration and respiration

$$L_{nw} = \frac{m_0 - m_t}{m_0} \cdot 100, \tag{3.4}$$

where m_t – the batch mass at the time of evaluation (prior to sorting), kg.

The integral index of commercial yield reduction was calculated as the sum of the above components

$$I_{\Sigma} = L_{mic} + L_{phys} + L_{nw}. \quad (3.5)$$

Value I_{Σ} was presented in % and used as a comprehensive quantitative assessment of postharvest losses and the effectiveness of the applied treatments over the 40-day storage period.

The yield of standard products (Y_{std}, %) was calculated using the formula

$$Y_{std} = 100 - I_{\Sigma}. \quad (3.6)$$

The results of the experimental study and the calculated losses are presented in **Table 3.1**.

The results indicate a pronounced effect of the pre-cooling method on both the level and structure of postharvest losses in sweet cherry fruits, regardless of cultivar. After 40 days of cold storage, the integral loss index in the air-cooling variant (C1) ranged from 12.426 to 13.597%, reflecting a typical decline in product quality under intensive air cooling without additional stabilization measures. In this variant, natural weight loss predominated (5.2–5.4 %), while losses due to microbiological damage and physiological disorders accounted for 3.8–4.8% and 3.4–3.8%, respectively. These findings highlight the dominance of transpiration-driven water deficit under conventional air cooling.

In the hydrocooling variant without acids (C2), the integral loss index increased to 13.836–15.470%, representing the highest values among the tested methods. Although weight losses were minimal (1.705–1.925%), the microbiological component increased substantially, reaching 7.684–8.913%, nearly double that of C1, while physiological disorder-related losses ranged from 4.025–4.841%. Surface wetting during hydrocooling reduced transpiration losses but created favorable conditions for microbial proliferation, resulting in a predominance of microbiological damage in the total loss structure.

The lowest integral losses were observed in the experimental variant (R), which combined lactic and acetic acids with a two-step cooling process that included the removal of residual surface moisture. Total losses were only 3.347–3.865%, 3.5–4.5 times lower than the control treatments. Microbiological losses decreased to 0.735–1.033%, physiological disorder-related losses ranged from 0.945–1.216%, and weight losses were 1.504–1.755%. The sharp reduction in the microbiological component (8–9 times lower than C2) confirms the efficacy of organic acids as

exogenous microbistatic agents. Concurrent air cooling effectively removed residual surface moisture, further limiting pathogen proliferation.

Table 3.1 Sweet cherry fruit losses after 40 days of storage

Cultivar	Treatment	Weight loss, %	Losses due to microbiological diseases, %	Losses due to physiological disorders, %	Integral loss index, %
Rubinova Rannia	C1	5.429 ± 0.249	4.285 ± 0.058	3.366 ± 0.154	13.080 ± 0.241
	C2	1.925 ± 0.045	8.913 ± 0.271	4.632 ± 0.193	15.470 ± 0.248
	R	1.755 ± 0.021	0.950 ± 0.017	1.071 ± 0.052	3.776 ± 0.041
Valerii Chkalov	C1	5.215 ± 0.226	3.822 ± 0.180	3.389 ± 0.097	12.426 ± 0.308
	C2	1.839 ± 0.060	8.401 ± 0.062	4.841 ± 0.039	15.081 ± 0.096
	R	1.739 ± 0.005	0.806 ± 0.003	1.068 ± 0.013	3.613 ± 0.009
Kazka	C1	5.412 ± 0.011	4.407 ± 0.072	3.605 ± 0.005	13.424 ± 0.065
	C2	1.876 ± 0.022	8.712 ± 0.101	4.619 ± 0.022	15.207 ± 0.076
	R	1.742 ± 0.009	0.999 ± 0.017	1.124 ± 0.047	3.865 ± 0.047
Talisman	C1	5.364 ± 0.089	4.106 ± 0.048	3.790 ± 0.098	13.260 ± 0.200
	C2	1.711 ± 0.013	7.732 ± 0.326	4.393 ± 0.073	13.836 ± 0.313
	R	1.511 ± 0.020	0.735 ± 0.009	1.101 ± 0.059	3.347 ± 0.069
Dilema	C1	5.445 ± 0.067	4.563 ± 0.220	3.442 ± 0.021	13.450 ± 0.245
	C2	1.721 ± 0.008	8.557 ± 0.044	4.032 ± 0.085	14.310 ± 0.089
	R	1.622 ± 0.024	1.033 ± 0.040	0.954 ± 0.037	3.609 ± 0.066
Melitopolska chorna	C1	5.427 ± 0.119	4.247 ± 0.196	3.617 ± 0.083	13.291 ± 0.240
	C2	1.705 ± 0.018	7.684 ± 0.073	4.539 ± 0.055	13.928 ± 0.090
	R	1.504 ± 0.056	0.909 ± 0.011	0.945 ± 0.011	3.358 ± 0.057
Karina	C1	5.225 ± 0.153	4.359 ± 0.226	3.566 ± 0.223	13.150 ± 0.185
	C2	1.811 ± 0.037	8.872 ± 0.062	4.025 ± 0.097	14.708 ± 0.109
	R	1.703 ± 0.081	0.918 ± 0.016	1.216 ± 0.063	3.837 ± 0.055
Regina	C1	5.387 ± 0.258	4.762 ± 0.140	3.448 ± 0.014	13.597 ± 0.316
	C2	1.823 ± 0.175	8.312 ± 0.133	4.444 ± 0.071	14.579 ± 0.212
	R	1.698 ± 0.082	0.961 ± 0.014	1.084 ± 0.072	3.743 ± 0.140
Krupnoplidna	C1	5.223 ± 0.061	4.274 ± 0.165	3.791 ± 0.193	13.288 ± 0.272
	C2	1.797 ± 0.141	8.057 ± 0.131	4.401 ± 0.041	14.255 ± 0.210
	R	1.689 ± 0.055	0.893 ± 0.038	1.092 ± 0.072	3.674 ± 0.089

Cultivar differences were moderate and did not alter the overall pattern of the technological factor's effect. The lowest integral losses in the experimental variant were observed for the Talisman and Melitopolska chorna cultivars, which may be associated with the morphological features of the skin and tissue density. At the same time, the effect of the cooling method substantially exceeded inter-cultivar variation, indicating the decisive role of technological treatment in determining postharvest stability.

A two-way analysis of variance of the integral loss index after 40 days of storage (**Table 3.2**) revealed a statistically significant influence of the cooling method ($F(2,108) = 48{,}517.07$; $p < 0.001$), cultivar ($F(8,108) = 25.80$; $p < 0.001$), and their interaction ($F(16,108) = 23.06$; $p < 0.001$). The total sum of squares was $SS_t = 3{,}223.8861$ ($df = 134$), with the main contribution to variation arising from the cooling method factor ($SS_A = 3{,}201.3415$), while the contributions of cultivar ($SS_B = 6.8100$) and the interaction ($SS_{AB} = 12.1715$) were considerably smaller. The mean square error was $MSE = 0.0330$ ($df = 108$).

The effect size confirms the dominance of the technological factor: η^2 for the cooling method was 0.9930, indicating a decisive influence of the applied technology on loss formation. The contribution of the cultivar factor was considerably lower ($\eta^2 = 0.00211$), yet statistically significant, while the significant interaction between factors reflects the cultivar-specific nature of fruit responses to different cooling methods.

These results confirm that the formation of the integral loss index is primarily determined by the technological regime, whereas cultivar-specific traits modulate – but do not dictate – the postharvest stability of the fruits.

The dominant role of the cooling method, as established by the two-way ANOVA, is also clearly reflected in the structure of the standard product yield at the end of the storage period (**Fig. 3.1**).

Table 3.2 Two-way ANOVA results

Source of variation	*SS*	*df*	*MS*	*F*	*p*-value	F_{crit} (0.05)	η^2
Cooling method (A)	3201,342	2	1600,671	48517,07	$3.05 \cdot 10^{-160}$	3.0804	0.9930
Cultivar (B)	6.810	8	0.851	25.80	$7.43 \cdot 10^{-22}$	2.0252	0.00211
A × B	12.172	16	0.761	23.06	$1.10 \cdot 10^{-27}$	1.7380	0.00378
Residual	3.563	108	0.033	–	–	–	0.00111
Total	3223,886	134	–	–	–	–	1.0000

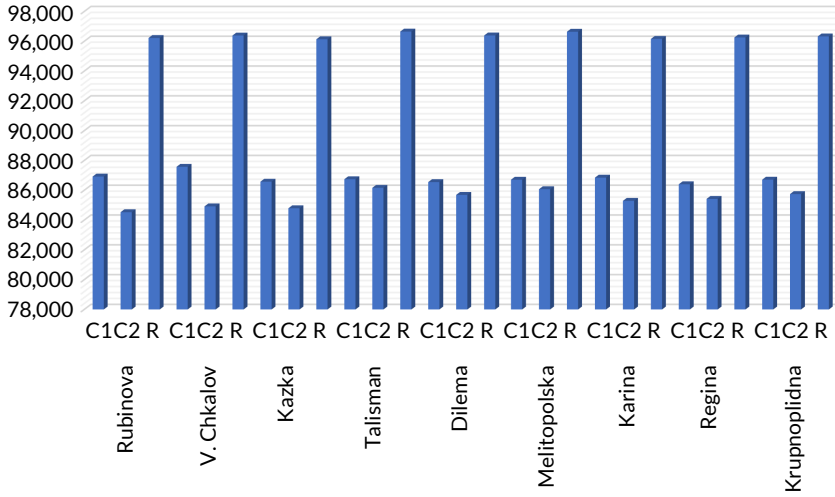

Fig. 3.1 Yield of standard sweet cherry fruits after 40 days of storage, %

The highest yield of standard fruits was observed in the experimental variant (R), which employed combined cooling with the use of an exogenous protective composition. For all cultivars studied, the yield exceeded 96%, with several cultivars approaching 97%.

Cultivar-specific differences in the absolute values of standard product yield persisted; however, their amplitude was substantially smaller compared to the effect of the cooling method.

These results demonstrate that the application of an exogenous acid composition within a combined cooling scheme ensures a consistently high yield of standard products regardless of cultivar, which is critically important for producing a predictable commercial batch during prolonged storage of sweet cherries.

3.5 Mechanisms of oxidative homeostasis regulation in sweet cherry fruits under the influence of the exogenous protective composition after 40 days of storage

Postharvest cooling of sweet cherry fruits is a critical stage that determines the subsequent course of metabolic processes during storage. The initial method of heat removal establishes the physiological response of the tissues, which is reflected

in the patterns of redox regulation, the intensity of ROS formation, and the stability of membrane structures throughout the storage period.

Sweet cherries are high-respiration fruits with increased sensitivity to oxidative stress. Disruption of the balance between ROS generation and antioxidant system activity leads to intensified lipid peroxidation, destabilization of cellular membranes, and accelerated senescence. MDA is considered an integrative marker of lipid peroxidation and an indicator of membrane structural integrity.

The enzymatic antioxidant system in fruits includes SOD, which catalyzes the dismutation of superoxide anions into hydrogen peroxide; CAT and APX, which further detoxify hydrogen peroxide; and POD, involved in regulating redox reactions within the cell wall and apoplast. The coordinated activity of these enzymes forms a cascade system that maintains redox homeostasis.

The results of MDA content determination in experimental sweet cherry cultivars under different pre-cooling methods after 40 days of storage are presented in **Table 3.3**.

Table 3.3 Malondialdehyde content in sweet cherry fruits after 40 days of storage depending on cultivar and cooling method, nmol · g^{-1} FW

Cultivar	C1	C2	R
Rubinova Rannia	6.34 ± 0.33	7.21 ± 0.37	4.02 ± 0.21
Valerii Chkalov	6.18 ± 0.29	7.05 ± 0.32	3.89 ± 0.18
Kazka	6.46 ± 0.31	7.38 ± 0.35	4.11 ± 0.20
Talisman	6.07 ± 0.26	6.86 ± 0.28	3.62 ± 0.16
Dilema	6.22 ± 0.28	7.12 ± 0.31	3.84 ± 0.17
Melitopolska chorna	6.10 ± 0.25	6.90 ± 0.29	3.55 ± 0.15
Karina	6.28 ± 0.30	7.26 ± 0.34	4.07 ± 0.19
Regina	6.49 ± 0.34	7.44 ± 0.38	4.16 ± 0.22
Krupnoplidna	6.16 ± 0.27	7.01 ± 0.30	3.92 ± 0.18

After 40 days of storage, MDA content showed a clear dependence on the postharvest cooling method. In all studied cultivars, the highest MDA levels were recorded in the hydrocooling variant (C2), ranging from 6.86 to 7.44 nmol · g^{-1} FW. On average, this variant exceeded the lipid peroxidation intensity under air cooling (C1) by approximately 14–16%.

In the intensive air cooling variant (C1), MDA values ranged from 6.07 to 6.49 nmol · g^{-1}, indicating a moderate level of lipid peroxidation after 40 days of storage. Despite the absence of direct contact with water, this method did not

provide sufficient stabilization of membrane structures, as TBA reactive compounds levels remained considerably higher compared to the combined cooling variant.

The lowest MDA values across all cultivars were observed under the combined cooling method with the application of the exogenous protective composition (R), with levels ranging from 3.55 to 4.16 nmol · g^{-1}. On average, lipid peroxidation intensity in this variant was 60% lower compared to C1 and 80% lower compared to C2. Thus, the use of organic acids in the protective composition reduced the accumulation of lipid oxidation products by approximately 1.6–1.8 times relative to the control cooling methods.

It is important to emphasize that the pattern of changes was consistent across all studied cultivars, indicating a systemic mechanism of action of the exogenous composition, independent of genotypic characteristics. Even for cultivars with relatively lower MDA levels in the controls, the combined method reduced lipid peroxidation intensity by at least 40%.

The obtained results are consistent with the integral loss indicators, which is confirmed by a strong positive correlation between MDA content and total product losses ($r = 0.95$; $p < 0.001$). The coefficient of determination ($R^2 = 0.90$) indicates that about 90% of the variation in postharvest losses is explained by the intensity of membrane lipid peroxidation.

The observed differences in MDA levels reflect the integrative outcome of oxidative processes in the tissues; however, to elucidate the mechanisms underlying this effect, it is necessary to analyze the status of the enzymatic antioxidant defense system (**Table 3.4**).

After 40 days of storage, the activity of antioxidant enzymes in sweet cherry fruits strongly depended on the postharvest cooling method. In all studied cultivars, coordinated changes in SOD, CAT, APX, and POD activities were observed, reflecting differences in oxidative load and the capacity for detoxification of reactive oxygen species.

SOD activity, which catalyzes the conversion of superoxide anion to hydrogen peroxide, remained within a relatively narrow range in the control variants, whereas in the variant with the exogenous protective composition (R), it was consistently higher. Compared to intensive air cooling (C1), SOD activity increased moderately in the hydrocooling variant (C2) by 3–5 arbitrary units depending on the cultivar. In variant D, the increase was more pronounced – on average 13–16 arbitrary units relative to C1 – indicating a higher capacity of tissues to neutralize ROS at the end of storage.

Changes in CAT and APX activities confirmed this trend. In C2, CAT and APX activities were slightly higher than in C1, whereas in R, the increases were more substantial: CAT activity was higher on average by 2.5–3.5 μmol H_2O_2 · g^{-1} · min^{-1},

and APX by 0.6–0.8 μmol AA · g^{-1} · min^{-1} compared to C1. This indicates more efficient hydrogen peroxide removal and a reduced likelihood of its participation in lipid peroxidation reactions.

Table 3.4 Activity of antioxidant enzymes in sweet cherry fruits after 40 days of storage

Cultivar	Treatment	SOD, % inhibition	CAT, μmol H_2O_2 · g^{-1} · min^{-1}	APX, μmol AA · g^{-1} · min^{-1}	POD, μmol H_2O_2 · g^{-1} · min^{-1}
Rubinova Rannia	C1	41.82 ± 2.11	7.63 ± 0.42	1.62 ± 0.09	43.82 ± 1.93
	C2	45.33 ± 2.32	8.02 ± 0.51	1.73 ± 0.10	22.44 ± 1.12
	R	56.74 ± 2.54	10.41 ± 0.60	2.28 ± 0.12	19.59 ± 0.92
Valerii Chkalov	C1	40.93 ± 1.89	7.38 ± 0.42	1.58 ± 0.08	45.26 ± 2.01
	C2	44.13 ± 2.22	7.83 ± 0.44	1.70 ± 0.09	25.66 ± 1.33
	R	55.41 ± 2.41	10.12 ± 0.56	2.22 ± 0.11	20.13 ± 1.08
Kazka	C1	42.61 ± 2.07	7.73 ± 0.55	1.66 ± 0.09	40.16 ± 1.82
	C2	46.05 ± 2.47	8.14 ± 0.46	1.78 ± 0.10	21.38 ± 0.93
	R	57.82 ± 2.63	10.61 ± 0.67	2.34 ± 0.12	18.18 ± 0.72
Talisman	C1	39.83 ± 1.84	7.16 ± 0.42	1.52 ± 0.08	38.27 ± 1.65
	C2	43.07 ± 2.18	7.52 ± 0.41	1.64 ± 0.09	17.48 ± 0.82
	R	53.96 ± 2.37	9.74 ± 0.52	2.12 ± 0.10	20.31 ± 0.94
Dilema	C1	41.27 ± 1.92	7.52 ± 0.44	1.60 ± 0.08	41.18 ± 1.74
	C2	44.63 ± 2.21	7.95 ± 0.53	1.72 ± 0.09	17.37 ± 0.82
	R	55.81 ± 2.47	10.27 ± 0.61	2.24 ± 0.11	14.15 ± 0.72
Melitopolska chorna	C1	40.13 ± 1.82	7.24 ± 0.44	1.54 ± 0.08	42.15 ± 1.62
	C2	43.45 ± 2.11	7.64 ± 0.42	1.66 ± 0.09	17.43 ± 0.85
	R	54.19 ± 2.32	9.84 ± 0.45	2.14 ± 0.10	20.13 ± 0.93
Karina	C1	41.6 ± 2.0	7.63 ± 0.55	1.61 ± 0.09	40.22 ± 1.82
	C2	45.1 ± 2.3	8.04 ± 0.57	1.74 ± 0.10	18.56 ± 0.87
	R	56.4 ± 2.5	10.35 ± 0.61	2.26 ± 0.12	15.32 ± 0.78
Regina	C1	42.93 ± 2.16	7.83 ± 0.52	1.67 ± 0.10	48.63 ± 2.17
	C2	46.43 ± 2.42	8.24 ± 0.57	1.80 ± 0.10	22.96 ± 1.17
	R	58.29 ± 2.74	10.73 ± 0.61	2.36 ± 0.13	20.25 ± 1.08
Krupnoplidna	C1	40.73 ± 1.94	7.32 ± 0.45	1.57 ± 0.08	40.13 ± 1.72
	C2	44.04 ± 2.26	7.68 ± 0.42	1.69 ± 0.09	16.25 ± 0.81
	R	55.13 ± 2.47	10.03 ± 0.64	2.20 ± 0.11	14.13 ± 0.74

The most pronounced differences were observed for POD. In C1, its values were the highest, ranging from 38 to 49 μmol $H_2O_2 \cdot g^{-1} \cdot min^{-1}$ depending on the cultivar. In C2, POD activity decreased to 16–26 μmol $H_2O_2 \cdot g^{-1} \cdot min^{-1}$, and in R to 14–22 μmol $H_2O_2 \cdot g^{-1} \cdot min^{-1}$. Thus, compared to intensive air cooling, hydrocooling and the combined method with the exogenous composition reduced POD activity 1.7–2.5-fold.

Considering POD localization in the cell wall and apoplast, these differences can be linked to varying intensities of oxidative processes in the extracellular space. High POD activity in C1 corresponds to dehydration stress and more intense oxidative reactions under active air circulation. The reduced POD activity in C2 and R indicates lower apoplastic oxidative stress.

Overall, the enzymatic profile after 40 days of storage shows that combined cooling with an exogenous protective composition is accompanied by increased SOD, CAT, and APX activities while simultaneously reducing POD. This enzyme activity pattern aligns with decreased lipid peroxidation and minimal postharvest fruit losses.

To further clarify the role of the extracellular environment in these differences, apoplastic pH of fruit tissues was additionally analyzed after 40 days of storage (**Table 3.5**).

Table 3.5 Apoplastic pH of sweet cherry fruits after 40 days of storage

Cultivar	C1	C2	R
Rubinova Rannia	4.18 ± 0.05	3.86 ± 0.04	3.52 ± 0.03
Valerii Chkalov	4.22 ± 0.06	3.90 ± 0.05	3.55 ± 0.04
Kazka	4.15 ± 0.04	3.82 ± 0.04	3.48 ± 0.03
Talisman	4.10 ± 0.05	3.78 ± 0.03	3.46 ± 0.03
Dilema	4.17 ± 0.05	3.84 ± 0.04	3.50 ± 0.03
Melitopolska chorna	4.12 ± 0.04	3.80 ± 0.04	3.47 ± 0.03
Karina	4.16 ± 0.05	3.83 ± 0.04	3.49 ± 0.03
Regina	4.24 ± 0.06	3.91 ± 0.05	3.56 ± 0.04
Krupnoplidna	4.14 ± 0.05	3.79 ± 0.04	3.45 ± 0.03

After 40 days of storage, the apoplastic pH in the variant with the exogenous protective composition was consistently 0.6–0.7 units lower compared to intensive air cooling. Intermediate values were observed in the hydrocooling variant. This pattern was consistent across all studied cultivars.

The decrease in extracellular pH corresponds with the reduced POD activity in variant R and indicates less pronounced oxidative processes in the cell wall and apoplast. A more acidic microenvironment may limit the intensity of peroxide-dependent reactions and create less favorable conditions for the development of microbiological damage, which correlates with the minimal integral losses of the product.

Thus, in addition to the increased activity of intracellular detoxifying enzymes (SOD, CAT, APX), the reduction of apoplastic pH in the variant with the exogenous composition acts as an additional mechanistic factor for maintaining sweet cherry fruit quality during prolonged storage.

3.6 Conceptual summary of biochemical mechanisms for maintaining sweet cherry fruit quality under the influence of an exogenous protective composition

The study demonstrated that the use of a combined postharvest cooling method with an exogenous protective composition significantly reduced oxidative stress in sweet cherry fruit tissues after 40 days of storage compared to the control variants of intensive air (C1) and hydrocooling (C2). This is evidenced by the lower content of MDA – a key marker of membrane lipid peroxidation – in variant R, whereas its accumulation was significantly higher in the C1 and C2 controls. The reduction of TBARS-products in the composition-treated variant indicates stabilization of membrane integrity and limitation of oxidative stress, which aligns with the well-established role of MDA as an integral indicator of lipid peroxidation [21].

Analysis of the enzymatic response of the antioxidant system revealed coordinated changes in the activity of SOD, CAT, APX, and POD depending on the cooling method. In variant R, the highest activities of SOD, CAT, and APX were recorded, indicating an enhanced capacity of the tissues to neutralize reactive oxygen species. As is well known, SOD catalyzes the dismutation of superoxide anion into H_2O_2, while CAT and APX ensure further detoxification of hydrogen peroxide, preventing its accumulation and the initiation of lipid peroxidation reactions. Similar patterns have been reported in postharvest physiology studies, where increased activity of antioxidant enzymes is associated with reduced MDA levels and improved preservation during cold storage [22].

Notably, POD activity was highest in variant C1, where oxidative stress was more pronounced, whereas in variants C2 and R, its level was substantially lower. Considering the POD localization in the apoplast and cell wall, these differences reflect

the varying intensity of extracellular oxidative processes. Additionally, in variant R, the apoplastic pH was lower compared to the controls, which could have modified the activity of cell wall-associated enzymes and limited the progression of peroxide-dependent reactions. It is known that apoplastic acidity influences the intensity of redox processes and the functioning of the peroxidase system, which is consistent with the obtained results [21].

The integration of biochemical and physiological indicators demonstrates a clear pattern: the control variants with intensive air and hydrocooling exhibited higher oxidative stress (elevated MDA and POD levels) and greater postharvest losses, whereas the combined variant with the protective composition ensured minimal accumulation of lipid peroxidation products with an optimized enzymatic response and the lowest product losses. This relationship between biochemical markers of oxidative stress and fruit quality indicators aligns with international studies, where activation of the antioxidant system is considered a key mechanism for prolonging storage life [23, 24].

Considering the obtained experimental and calculated data, the practical significance of the proposed technology acquires a clearly defined economic value. The application of the combined cooling method with an exogenous protective composition ensures the reduction of integral postharvest losses to a minimal level (around 3–4%), directly increasing the yield of standard products and the proportion of marketable fruits.

With marketing 1 ton of cooled cherry fruits, revenue amounts to 23,000 UAH (550 EUR), while the additional net profit from implementing the proposed technology increases by almost 12,920 UAH per ton of product (307–308 EUR). The socio-economic effect from increasing the yield of standard products during storage using the combined method amounts to 2,918.2 UAH per ton of fruits (69–70 EUR).

Thus, the biochemical stabilization of oxidative homeostasis through the use of the exogenous protective composition affects not only the improvement of the physiological state of the tissues but also produces a measurable economic outcome. The combined cooling technology enhances the profitability of cherry fruit storage and strengthens their competitiveness within extended logistical chains and market distribution.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper.

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Data availability

Manuscript has no associated data.

Use of artificial intelligence statement

The authors used the AI assistant Perplexity (Grok 4.1, Perplexity AI) for translation and literature source selection. The authors bear full responsibility for the final manuscript. Generative AI tools are not credited and are not responsible for the final results.

Authors' contributions

Olesia Priss: Supervision, Conceptualization, Methodology, Writing – original draft, Investigation, Project administration.

Iryna Ivanova: Conceptualization, Methodology, Writing – original draft, Investigation.

Marina Serdyuk: Methodology, Writing – original draft, Writing – review and editing, Investigation, Visualization, Validation.

Tetiana Tymoshchuk: Writing – original draft, Writing – review and editing, Visualization, Formal analysis, Validation.

Olga Pyurko: Writing – original draft, Visualization, Formal analysis, Validation.

Sergii Basanets: Resources, Formal analysis, Visualization.

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CHAPTER 4

Evaluation of the usage of spontaneous fermentation sourdough starters and their influence on the quality indicators of wheat bread

Nataliia Slobodyanyuk
Oksana Tkachuk
Tetiana Brovenko
Tetiana Lebedenko
Halyna Tolok
Tamara Novichkova
Petro Drozd
Mykola Gruntkovskyi

Abstract

The limited availability of production laboratories at mini-enterprises, as well as outdated regulatory approaches to quality control which do not fully meet the modern range of bakery products, technological solutions, and consumer demands, significantly complicate the implementation of innovative technologies in the industry.

The aim of this study was to evaluate the feasibility of using and the effect of spontaneous fermentation sourdough, prepared in two different ways (dry and liquid starters based on wine yeast) on the quality indicators of wheat bread.

In the process of forming requirements to the quality of wheat bread, the physical, chemical, and biotechnological indicators of dry and liquid sourdough were determined; their sensory characteristics were evaluated.

As a result of the test baking, the quality indicators of wheat bread made using spontaneous fermentation starters based on wine yeast were evaluated.

Bread samples prepared with 5% and 7% of sourdough to the weight of flour, and control bread prepared without sourdough were compared in terms of shelf life and sensory properties. The dough preparation technology provides for a leaven-free method, with thick and liquid leaven; control samples were prepared using traditional technology. The addition of sourdough significantly affected the specific volume and porosity and slowed the loss of moisture during storage. Sensory

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properties improved compared to the control samples. Using 7% of liquid sourdough gave the most effective results.

The obtained results substantiate the technological feasibility of using the studied spontaneous fermentation sourdough starters in the production of bread products and demonstrate their potential for improving their quality indicators. Technologies and methodologies are recommended for mini-enterprises and craft bakeries.

Keywords

Bread products, sourdough starters, wine yeast, spontaneous fermentation, quality indicators.

4.1 Introduction

Bakery products for many nations of the world are synonymous with well-being, national wealth and food security, they have long accompanied a person from the first days of birth and throughout life as the most stable source of energy and nutrients and biologically active substances. Bread, its recipe and cooking technology are one of the points of national identification, an integral part of history, traditions and culture [1]. However, over the past half century, the US and EU countries have seen a decrease in the consumption of primarily traditional bakery products, and there is a growing trend towards a gluten-free diet [2] This is associated with the inconsistency of sensory characteristics, physical and chemical indicators, safety, and product range with consumer demands [3].

Quality criteria that cause concern also include the low content of nutrients and biologically active substances in significant volumes of products; high calorie content and amount of easily digestible carbohydrates; significant amount of salt; the use of a wide range of synthetic additives at all stages of industrial production (growing grain, obtaining flour, making bread); wheat and products made from it are among the top ten allergens, including due to gluten; the threat from yeast and genetically modified organisms; high content of acrylamide, which is formed during bread making and has carcinogenic properties, etc. All this raises doubts among consumers and a number of nutritionists about the safety of bread and fears of the spread of a whole range of diseases: diabetes, celiac disease, allergies, depression, cardiovascular diseases, cancer, metabolic disorders, gastrointestinal tract, nervous system [4].

In this regard, research has been intensified to study the nutritional characteristics of bread and the factors of their formation and influencing factors and to find answers to the question: "Why could our ancestors consume and digest bread,

but for today's consumers this is a problem?" One of the reasons is the transition to industrial accelerated preparation of dough and bread using active commercial yeast, the loss of the traditions of long-term dough preparation with sourdough, including spontaneous fermentation [5].

The popularity of craft bread is growing since it uses long-term fermentation technologies which ensures the formation of strong bread sensory characteristics, the transformation of biopolymers with improved digestibility, storage stability without the use of synthetic improvers, etc. [5, 6].

In times of crisis during the pandemic, hostilities, destruction of infrastructure, restrictions on logistical connections, rising energy prices and blackouts, craft and artisanal bakeries and technologies for long-term dough preparation using local raw materials have gained special importance. They play an important role in ensuring accessibility to basic food products due to their flexibility, high level of adaptability, and proximity to the raw material producer and consumer [7].

Restoration of ancient national culinary traditions, the use of technologies that were formed under the influence of history, culture, available raw materials, nature and climate can help in solving the problems of production and consumption [8].

4.2 History and traditions of bread baking: the basis for the formation of scientific principles for integrating wine yeast into spontaneous sourdough bread technology for sustainable production of quality products

For the sustainable development of modern bread baking in European countries, five associations (AIBI – Association of Plant Bakers; CEBP – European Confederation of national Bakery and Confectionery Organizations; COFALEC – Confederation of European Yeast Producers; European Flour Millers; Fedima – Federation of European Manufacturers and Suppliers of Ingredients to the Bakery, Confectionery and Patisseries Industries), which unite small, medium and large manufacturers of bakery products, flour mills and manufacturers and suppliers of yeast and other ingredients, created a coalition in Brussels in 2016 and signed a Memorandum on the formation of the "Bread Initiative".

The main tasks are to revive the history and introduce innovations in bread baking, declare bread and its technology as UNESCO World Intangible Cultural Heritage, debunk myths and unfounded stereotypes about the harm of bread products to the human body, and support developments aimed at creating a range of products that will better satisfy the views of consumers, doctors, and producers [9].

As part of the "Bread Initiative" project, a program document "Let's Keep Bread on the Table" was prepared and adopted, which proposed key measures for 2024–2029 to implement the adopted strategy for the development of bread baking to maintain the image and improve the production of bread as a nutritious and tasty staple food in the diet of Europeans, guarantee sustainable production and food security, taking into account the challenges of modernity, particularly the pandemic [10].

Therefore, one of the tasks of the European "bread initiative" is to preserve the cultural heritage associated with bread, collect information on the history of bread baking, which will allow to offer measures proven over the centuries to improve the quality and nutritional value of products. For example, it is possible to preserve and enhance the valuable physiological properties of grain and improve the consumer characteristics of bread, reduce the risk of its allergenic properties as a result of a better use of cereals by returning to ancient crops such as spelt that have not undergone selection and genetic changes [11], as well as by reviving ancient recipes and technologies of bakery products traditional for different peoples, which involve long-term ripening of dough and the use of sourdough starters with natural microflora [12]. Consumers, doctors, and specialists around the world note the relevance of studying the centuries-old experience of making bread in the context of eras, territories and peoples, establishing promising technological techniques in terms of effective comprehensive formation of product quality. This coincides with the statements of the European "Bread Initiative" that the revival of ancient national technologies should become the basis of an alternative strategy for the development of modern bread baking.

But this is an extremely difficult, global and multidisciplinary task, since the information is largely lost and ambiguous, and thus requires search and restoration, conducting modern research and analysis of archaeological finds, historical references, documentary sources and ethnography of different peoples, and their systematization with the participation of specialists from various fields.

The generally accepted history of bread baking, which has been adhered to for a long time, is as follows. Cereals were used by man as a food product as early as 7000 BC. The first loaves of bread were found in the Neolithic period, which are from 6000 to 9000 years old. According to one hypothesis, sourdough, as a mixture of flour and water, fermented by natural lactic acid bacteria and wild yeast, was first used to leaven bread in Ancient Egypt, about 4000 BC. From Egypt, the art of bread baking, according to this hypothesis, spread north to Ancient Greece, where bread was also the main product, and more than 70 different savory and sweet types were produced. References to this date back to the 5th century BC. The Romans adopted the technology of sourdough bread production and spread it throughout Europe.

According to the works of the ancient Roman historian Pliny the Elder, sourdough bread was widespread in the Roman Empire in the 2nd century BC [13].

According to another hypothesis, which appeared much later, the first traces of yeast bread come from Europe, when it appeared in the 5th millennium BC. During this period, agriculture and grain cultivation flourished from the Balkans to Ukraine, and remains of leavened bread have been found in Romania, in the Danube Delta, in the Swiss Alps, and elsewhere in Europe [14].

Recent studies of the yeast genome have shown that the canonical brewer's and baker's yeast *Saccharomyces cerevisiae* originated in China and, according to another hypothesis, it was from there that they spread to the West along the Silk Road 16–14 thousand years ago [15].

In recent years, the database on the evolution of cereals and bread has been expanding. Thus, modern archaeological and botanical studies indicate that cereals have been used by humanity for more than 14 thousand years [16], first beer was produced about 13 thousand years ago, and the first bread from yeast-free (unleavened) dough was made about 12 thousand years ago. Genetic studies of wine residues have given grounds to assume that it was the microflora from the surface of grapes that was used approximately 8500–4000 BC. in winemaking, bread making and even for grain fermentation in beer brewing and preparation of other fermented beverages. Such a culture, according to the authors [17], was traditional for the Middle East, Mesopotamia, the Black Sea region (Trypillian culture, Caucasus, Transcaucasia, Eastern Turkey, Ancient Italy, and other countries), where grapes were grown.

The Gauls (ancient tribes in what is now France, Belgium, parts of Switzerland, Germany, Romania, and Northern Italy) and the Iberians (in modern Spain) used foam skimmed from beer when making dough. Some sources attribute the Celtic people to the first use of beer brewing by-products to simplify bread making. British texts from the 11th century mention the use of dried foam, called "*barm*", which appeared on the surface of the fermenting liquid used in the production of traditional ale. According to the authors, this method became widespread in Europe in the 14th and 15th centuries [13].

Sourdough continued to be used in Europe, especially for the production of rye bread, which requires high acidity. Rye sourdough, including based on spontaneous microflora, is still widely used for dough preparation in the countries of Northern and Eastern Europe, where a lot of rye bread is traditionally consumed.

As for the Ukrainian history of bread baking, there is little information as well. It is known that the beginning of grain farming, the cultivation of wheat and barley in Ukraine dates back to the 7th millennium BC, it is associated with the Trypillia culture. The ability to cook unleavened bread is indicated by clay models of loaves,

which were discovered in settlements of the 6th millennium BC. Barley grains that underwent fermentation dated to the 5th millennium BC have been found, which proves the ability to make beer. In settlements of the Black Sea region of the same period, grape seed prints were found, which indicates the possibility of winemaking in this territory. That is, the preparation of alcoholic drinks begins about 7 thousand years ago [18, 19].

This is the basis for the assumption that under such conditions it is possible to use wine and beer yeast to prepare "fermented" ("yeast", "sour", "leavened") dough, but archaeological, documentary evidence for this has not been found. There is no unanimous opinion among scientists regarding the source of the dough leavening method in Ukraine, and the exact time frame for the beginning of its use in bread making has not been established. The appearance of sourdough bread in various sources [20] dates from the 3–4th centuries AD, which is hypothetically explained by borrowing the term "hlaifs" of Gothic origin for the name of leavened bread, to the 6–7th centuries AD, confirmed by the finds in Slavic settlements of ritual clay loaves that imitated baked leavened loaves. Although, given the close ties with Ancient Greece, the undeniable existence in the Northern Black Sea region of the Greek city-states of Tyre, Olbia and others, founded as early as the 7–5th centuries BC, it is worth noting the assumption that the spread of the traditions of leavened bread came from here and the term "bread" may come from the Ancient Greek word "klibanos": the name of a conical baking dish.

However, documentary references to baking leavened (sourdough) bread date back to the times of Kievan Rus (11th century), which prove the use of various types of leavens in Orthodox churches, monasteries and households [21]. According to the famous Ukrainian ethnographer, academician M. Sumtsov, the invention of "sourdough" bread occurred as a result of the fact that the dough, which remained in an uncleaned vessel, began to ferment and turned into sourdough, which induced fermentation in the new dough [22].

Sourdough recipes and methods of making them have varied somewhat across the regions of Ukraine and over time. They were made from rye or wheat flour, less often mixed with buckwheat or corn flour, with the addition of water and possibly hop decoction, yarrow, whey, sour milk, brewer's yeast, wine must, bean broth, boiled beets, potatoes, etc.

The process of making sourdough bread was complex and time-consuming. The bread was kneaded in a wooden trough or in a bowl, kneading a portion of the flour with warm water and adding "roshyna" (a piece of dough left over from the previous preparation of bread), "rozkryshka" (a loaf of bread kneaded in hop water) or another type of microbiological and biochemical starter. Enrichers of the water-flour mixture

were also added, which created better conditions for the reproduction of fermenting microflora. For example, sometimes the dough was made with "grits" (steamed rye bran that had fermented); in some villages, tartar or boiled potatoes were added. This steamed mixture, called "prima", was left to ferment in a warm place. When the mass began to ferment, it was kneaded, adding the main flour. The dough was kneaded for a long time, until it began to peel from the walls of the vessel and from the hands. After that, it was left in a warm place again to rise, then kneaded again and made into loaves. Ukrainians baked leavened bread from rye, wheat, oat or barley flour. In the Hutsul region, it was also prepared from corn flour, and sometimes rye flour was mixed with corn or barley flour [18].

Significant changes in the preparation and quality of products are associated with a new stage in the development of world baking in the 19th century, namely with the improved process of obtaining yeast. Then the first yeast factories were created, which facilitated the work of baking enterprises, but caused significant damage to the technology of sourdough bread. The use of commercial yeast allowed to reduce the duration to 5–12 hours and stabilize the bread baking process.

In 1961, scientists at the Chorleywood Flour Milling and Bakery Research Association Laboratories in Hertfordshire, UK, developed a new industrial process for rapid mass production of bread. Bread could now be made in just three and a half hours, from flour to finished product, reducing the lengthy fermentation process to a minimum. To produce acceptable quality bread in the shortest possible time, Chorleywood technology required not only the use of special high-speed dough mixers, but also a whole arsenal of additives: additional yeast or their highly active strains, gluten, fat to improve the softness of the crumb, reducing agents to obtain a more elastic dough, soy flour for better volume and softness, emulsifiers to slow down staling, preservatives to extend the shelf life and various enzymes that are not legally required to be indicated on the label. As noted in a 1974 report by the Center for Consumer Technology Assessment, British bread is considered the most chemically processed in Western Europe [23].

This technology spread throughout the world. Mass production of bread drove many bakeries out of business, leaving only large bread factories. But at the same time, the quality of the products steadily declined, which became the root cause of the constant decline in bread consumption.

At the end of the last century, in various countries of the world (France, Italy, the northern countries of Europe and others), when manufacturers faced the above-mentioned problems of a long-term and significant decline in demand for their products, a "retro-innovation" movement (the restoration of ancient traditions) was launched.

For Ukraine, the revival of ancient recipes and technological techniques for making bread, the study of centuries-old culture, wisdom and craftsmanship of our people, the basis of Ukrainian cuisine, combined with the wealth of our soil, nature and some of the oldest traditions of agriculture, can become the basis for the development of retro-innovative bread technologies.

Such technologies include the preparation of dough with wine yeast. This technology is widespread in the southern regions of Ukraine [24] and was included as a unique ingredient in baking and as a valuable gastronomic heritage in the atlas "Ark of Taste of Ukraine".

The issues of the revival of regional bread-making traditions and their adaptation to modern conditions were addressed by such domestic scientists in the field of bread-making as V. Drobot, V. Yurchak, V. Rak, O. Naumenko, G. Pshenyshnyuk, T. Sylchuk, L. Mykhonik, V. Chelyabieva, S. Mykolenko, N. Sokolova, N. Getman, T. Semko and others, ethnographers S. Tvorun, L. Artyukh, N. Sumtsov, A. Zyubrovsky, M. Glushko, S. Tsypishev and other researchers.

A typical feature of ancient national bread-making technologies is the long preparation of dough using spontaneous starters. Wheat, rye or other types of flour were used to produce them; brewer's and wine yeasts or other carriers of fermentation microbiota were added for faster formation of the specified technological properties; hop extracts, spicy and aromatic plant additives, etc. were added to control the species composition of the starter microbiome.

Bread technologies based on spontaneous starters have a number of advantages, which are the reason for the growing interest in them from consumers, producers and researchers:

1) the formation of strong taste and aroma;

2) improvement of the structural and mechanical properties of the crumb, its elasticity and texture;

3) shelf life extension, elimination of excessive brittleness;

4) increase of microbiological stability during storage;

5) improvement of the functional and physiological properties of bread: digestibility and bioavailability of nutrients and biologically active substances, reduction of the allergenic effect of gliadin, glycemic index, etc.

However, the production of bread based on spontaneous fermentation, in addition to the above-mentioned advantages, also has problems that hinder its implementation. There is limited information in this area regarding recipes and technological process of sourdough development, its management in production, dough preparation, the lack of clear requirements for the quality of raw materials, technological properties and microbiome of sourdough starters, semi-finished

products based on them, as well as sensory characteristics, physical, chemical, functional and physiological indicators of finished products. There are no recommendations on effective methods for assessing raw materials, semi-finished and finished products and controlling the flow of the technological process, which ensures the formation of a given quality of products. The scientific research is aimed at solving these problems.

Effective integration of wine yeast into bread baking technology requires a comprehensive scientific justification of the stages of their preparation. The key task is to optimize the process of obtaining dry wine yeast, taking into account their further functioning in the dough.

Particular attention is required to establish and systematize data on the influence of factors of raw material origin: grape variety, region and agroclimatic conditions of cultivation on the formation of physiological and biochemical properties of yeast cultures. No less important are the parameters of production of dry wine yeast (cultivation, drying, stabilization regimes), which determine their fermentation activity, stress resistance, enzymatic potential and ability to adapt to the environment of wheat dough.

Systematic analysis of these factors allows predicting technologically valuable characteristics of wine yeast in the context of baking production and forms the basis for developing standardized approaches to their use as part of traditional and sourdough technologies.

The purpose of the research was to assess the feasibility of using and the impact of spontaneous fermentation starters, prepared in two different ways (dry and liquid sourdough based on wine yeast) on the quality indicators of wheat bread.

4.3 Raw materials and factors of formation of physiological, biochemical and technological properties of wine yeast

Grapes, as the main ingredient for obtaining wine yeast, are characterized by a complex morphological chemical organization. Its component composition and microbiological characteristics determine the technological properties, the course of fermentation processes and the quality indicators of the final product. Systematic analysis of the mechanical and chemical composition and microbiota of grapes is a necessary prerequisite for substantiating technological processing modes and predicting biochemical activity in fermentation systems of starter cultures.

The chemical composition of grapes is a multicomponent and dynamic system, which is formed under the influence of genetic, environmental and agrotechnological

factors. The composition of grapes includes water, carbohydrates, dextrans, plant gums, pectins, organic acids, phenolic compounds (tannins and dyes), aromatic components, lipids, waxes, nitrogenous compounds, enzymes, vitamins and mineral elements. The distribution of these components within fruit is uneven, which is due to the physiological specialization of its structural elements (pulp, peel, seeds, ridges). Quantitative and qualitative indicators of the chemical composition of grapes vary depending on the variety, soil and climatic conditions of cultivation, meteorological features of the growing season, agrotechnical techniques, phytosanitary conditions of the plantations and the mineral nutrition level of the plants.

The ratio of mechanical and plastic elements of grapes (pulp, peel, seeds, ridges) is a specific characteristic determined by environmental factors and affects the technological suitability of the ingredients. The mass of the grape cluster, the number and mass of fruits, the mass of peel and seeds, as well as the number of seeds are experimentally determined, which allows to assess the mechanical composition and predict the yield of the must and the concentration of extractive substances.

The pulp makes up an average of 85–90% of the fruit mass and is the main source of must. In addition to water, grape juice contains monosaccharides: mainly glucose and fructose, as well as a small amount of sucrose. Pectic substances, localized in the cell walls in the form of protopectin, pass into a soluble state during crushing and maceration of berries, affecting the viscosity and colloidal stability of the must. The second place in technological importance after sugars is occupied by organic acids. They are represented by tartaric and malic acids as dominant components, as well as citric acid and trace amounts of glycolic and glucuronic acids. Organic acids are in free, semi-bound and bound states, forming the buffer properties of the system and determining the titrated acidity of the must.

Nitrogenous substances are mainly represented by low-molecular compounds (amides, amines and ammonium salts), which are available as sources of nitrogen for yeast in the process of alcoholic fermentation. Despite the relatively low concentration, enzymes, vitamins and minerals play a significant role in the formation of the biochemical potential of the must, ensuring the metabolic activity of the microbiota and the stability of fermentation processes. The composition of the juice in the central and peripheral zones of the pulp is heterogeneous, which should be taken into account when assessing extractability and predicting fermentation kinetics.

The peel makes up an average of 9–11% of the fruit and is a concentrate of phenolic, coloring and aromatic compounds. It also contains nitrogenous and mineral substances, tartar, calcium oxalate and a waxy coating (pruin), which performs a protective function. The main component of the peel is water (60–80%). Coloring

substances (anthocyanins in red varieties) in fresh berries at normal temperatures are characterized by limited solubility in the aqueous medium. Therefore, with rapid pressing, it is possible to obtain a weakly colored or colorless must even from red varieties. An increase in temperature during alcoholic fermentation, as well as the accumulation of ethanol, contribute to the destruction of the cellular structures in the peel and increased solubility of phenolic compounds, which causes intensive extraction of dyes and tannins.

The distribution of phenolic and aromatic compounds in grapes and the physiological and biochemical properties of yeast cells are interrelated. These factors determine the kinetics of fermentation processes and the formation of sensory characteristics of grape processing products.

Grape aromatic compounds are localized mainly in the inner layers of the peel, adjacent to the pulp. During the mechanical destruction of grapes (crushing, pressing), they are extracted into the must, forming a variety-specific aromatic profile. The intensity of the transition of volatile components is determined by the degree of destruction of cellular structures, temperature regime, duration of contact of the pulp with the must, and physical and chemical parameters of the environment. Tannins (eno-tannins) are also concentrated in the deeper layers of the peel, bordering the pulp. Their content varies within 0.18–4.0% in fresh peel, 0.55–7.58% in dry peel, which determines the potential for the formation of astringency, structurality, and antioxidant properties of grape processing products [25].

The main substrate of alcoholic fermentation is must monosaccharides, which are metabolized by yeast of *Saccharomyces cerevisiae* species, which are unicellular microorganisms of ascomycetes class. Yeasts are naturally present on the surface of grapes, often visible as a light coating on the fruits.

Wine yeast is a product of the area, since each microzone has its own microflora. World producers are located in the largest wine-producing countries: France, Spain, Italy, Germany. Analysis of literary sources shows that the species composition of the microbiota of spontaneously fermented grape must was studied in the leading wine-producing countries of Europe, Asia and South America. According to studies conducted in 22 countries around the world, 93 different species of yeast were found on the surface of grape berries, 15 of which are involved in the formation of wine quality, the bacterial composition includes more than 50 species. Microorganisms of grape must at the beginning of fermentation are represented by yeast, mold fungi, lactic acid and acetic acid bacteria. The quantitative and qualitative composition of microorganisms in grape must can be very diverse and largely depends on the quality of grapes, the place and climatic conditions of cultivation, the sanitary condition of grapes and the technology of its [26].

The microbiota of grape must during fermentation changes under the influence of osmotic stress caused by high concentrations of sugars, acidic environment, anaerobic conditions, high concentrations of ethanol, low temperature, etc. In grape must during fermentation, yeasts of *Saccharomyces* species almost completely replace other microbiota due to different sensitivity to ethanol. Mold fungi, due to their sensitivity to alcohol, do not develop in the fermenting must. Wine yeasts of *Saccharomyces* species predominate in grape must at the end of fermentation: *S. vini* (60–90%), *S. oviformis* (6–10%), *S. paradoxus* (up to 3.4%), and *S. chevalieri* (up to 0.3%). Along with saccharomycetes, spontaneously fermented wine must may contain yeasts of other genera, including *Brettanomyces*, *Saccharomycodes*, *Zygosaccharomyce*, *Candida*, *Pichia*, *Hanseniaspora*, etc., which are also important for the final quality of wine. They affect oxidative reactions and can enhance the taste of wine, increasing the concentration of volatile compounds responsible for the fruity aroma [27]. This microbiota has aroused interest in the organization of dough fermentation for bread baking in terms of forming special sensory characteristics and physiological properties of the product.

For use in artisanal baking, yeast cultures must meet a set of technological requirements: have high fermentation energy (complete and rapid fermentation of monosaccharides), a predominantly anaerobic type of metabolism, resistance to elevated concentrations of ethanol, osmotic pressure and side metabolites, as well as adaptability to changes in the composition of the nutrient medium. When using yeast isolated from mature must, in baking technologies, their lifting power and maltase activity are additionally evaluated.

During the fermentation of grape must, foam is formed, which was selected to obtain wine yeast. The research used two samples of red grapes of the Zaiber variety, grown in the Bilhorod-Dnistrovskyi district of the Odesa region in 2023–2024.

For the preparation of the dough, premium wheat flour of TM "Zernari" trademark and 1st grade wheat flour TM "Zolote zernyatko" (GSTU 46.004-99), table salt (DSTU 3583:2015 "Table salt. General technical conditions. As amended"), and sugar (DSTU 4623:2023 "Sugar. Technical conditions") were used. For the control sample, pressed baker's yeast "Lvivski", manufactured according to DSTU 4812:2007 "Pressed baker's yeast. Technical conditions" was used. Premium wheat flour had average baking properties.

For saccharification of the leaven, unfermented barley malt was used in accordance with DSTU 4282:2018 "Brewing barley malt. General technical conditions". When conducting experimental studies, high grade wheat flour with average baking properties was used, which corresponds to GSTU 46.004-99 "Wheat flour. Technical conditions" (**Table 4.1**).

Table 4.1 Baking properties of wheat flour

Indicators	Premium wheat flour	First grade wheat flour
Flour moisture, %	12.3	13.8
Flour acidity, degrees	2.1	2.6
Amount of raw gluten, %	21.5	26.0
Elasticity on the VDK-1 device, units	95	70
Stretchability, cm	16	13.5
Gluten color	light	light
Gluten elasticity	good	good
Humidity, %	65	63
Hydration capacity, %	185	170
Amount of dry gluten, %	7.5	9.6

The sensory evaluation of flour was carried out according to standard methods described in the manual [28]. The following was determined: color – white; smell – typical of wheat flour, without foreign odors, not musty, not moldy; taste – typical of wheat flour, without foreign flavors, slightly sweet, without crunch.

4.4 Retro-innovative method of producing dry wine yeast based on spontaneous fermentation of grape must and evaluation of their biotechnological and sensory indicators for use in bread baking

Preparation method of dry wine yeast was as follows: foam was collected from the surface of grape must, where active fermentation was taking place, finely ground corn flour TM "Skvyrianka" was added to it and subjected to fermentation. After establishing active fermentation, more corn flour was added, the mass was formed into balls and dried in the shade. Dry wine yeast was stored in a bag made of natural fabric in a dry place.

Dry wine yeast (2 samples) was used as an unconventional ingredient for bread making, which was used to prepare dough semi-finished products. The dough was made without leaven, as well as with liquid and thick leaven where the dry "wine" yeast was pre-activated in 2 variants: in a mixture of flour and water and in brewed flour.

The studies used brewed wheat flour with a ratio of wheat flour (first and high grade) to water 1:4. The flour was brewed with water with a temperature of 88 ± 2°C. The duration of sugaring the brew was 2 hours.

Liquid "wine" yeast was prepared from dry "wine" yeast by carrying out the activation stage, the breeding cycle and maintenance with daily replenishment of their nutrient mixture of flour and water with 89–90% moisture for 15 days.

The dough was prepared without leaven and with liquid (W = 68–72%) and thick (W = 48–50%) leaven. When kneading the dough (W = 44–45%), salt and sugar solutions were added (1.3 and 3.0% of dry raw materials to the weight of flour respectively). The dough development, proofing of the loaves and baking of bread were carried out according to the technological instructions for products made from wheat flour.

The quality of semi-finished products was determined by sensory indicators and moisture content.

The moisture content in semi-finished products was determined by express drying on the Chizhov PCMC device according to the method [28]. The degree of maturation and readiness of liquid "wine" yeast and semi-finished products based on them were determined by the lifting force, which was determined by the rising of a dough ball according to the method and titrated acidity, which was determined by titration of a sample according to the method [28].

Bread quality indicators were determined 4–24 hours after baking. Determination of sensory quality indicators of the products was carried out according to DSTU 7044:2009. The moisture content in bread was determined by a standard accelerated method by drying in a SESH-2M cabinet. The bread volume was determined using an OHL device [28]. The crumb porosity was measured on a Zhuravlev device according to the method. The shape stability was measured on an IFK device [28].

To ensure scientifically substantiated introduction of wine yeast into baking production, it is necessary to solve a complex of technological and biotechnological tasks at the stage of obtaining dry forms of "wine" yeast. Of particular importance is the regulation of the sequence of their preparation, as well as the generalization and systematization of data on the effect of ingredients (grape variety, region of origin, soil and climatic conditions of cultivation, etc.) and production parameters of dry wine yeast on the formation of functional and technological properties.

The technology for preparing dry wine yeast consisted of successive stages.

Selection of foam from active fermentation of grape must. Foam was removed during the period of maximum gas formation intensity, which ensures a high content of metabolically active cells.

Mixing the nutrient medium. A mixture of wheat and corn flour is added to the foam as a source of starch, nitrogenous substances and minerals. Additional sugar stimulates the rapid start of the fermentation process due to invertase activity.

In some traditional practices, a small amount of ethanol is added, which selectively suppresses bacterial microflora and promotes the selection of alcohol-tolerant strains.

Fermentation occurs at a temperature of 34–36°C for 60–90 minutes. During this period, the following occurs: intensive yeast reproduction; biomass accumulation; partial hydrolysis of starch to fermented sugars; synthesis of secondary metabolites (higher alcohols, esters, organic acids), which form the aromatic profile.

Forming and drying. After the active fermentation is completed, the mass is formed into granular forms in the form of "sticks" or "sausages" and dried at room temperature (25–30°C). Drying was carried out under conditions of natural convection without direct insolation to a humidity that ensures microbiological stability [29]. A decrease in water activity inhibits cell metabolism and puts them into a state of suspended animation. It has been proven that *Saccharomyces cerevisiae* are able to maintain viability after dehydration due to the accumulation of trehalose and expression of stress proteins.

Storage. Dry cultures are stored in air-permeable containers (linen or cotton bags), which prevents moisture condensation and secondary microbial contamination.

As part of experimental research, dry forms of wine yeast were obtained based on spontaneously fermented must of red grapes of the Zaibe variety grown in the Bilhorod-Dnistrovskyi district of the Odesa region.

The sensory properties of dry wine yeast are presented in **Table 4.2**.

Table 4.2 Sensory properties of dry wine yeast

No. s/n	Technological processes, semi-finished products	Sensory properties
1	Grape must during fermentation	The liquid is opaque, the juice contains pulp and grape peel; the smell is clean with a pronounced varietal flavor, the taste is sweetish-tart. Color: dark burgundy; light yellow; pink/red (depending on the color of the grapes)
2	Grape foam, mixing with corn flour, fermentation	Light bubbly mass. On the surface there is a layer of pink bubbles, with inclusions of ground corn flour; the smell is clean with a pronounced varietal flavor. Color: burgundy yellow, pink yellow, light yellow
3	Adding corn flour, forming dry wine yeast	Balls containing corn flour; aroma of alcoholic and lactic fermentation. Color: uneven gray-burgundy, gray-pink, gray-yellow

Fermentation temperature was 34–36°C, duration was 60...90 minutes. Experimentally determined technological parameters of drying wine yeast: 20–32°C on a wooden surface. Drying the mixture to 16–17% moisture content ensured the production of dry wine yeast with stable characteristics.

Physical and chemical properties of dried wine yeast were studied (**Table 4.3**).

Table 4.3 Physical, chemical and biotechnological properties of dry wine yeast

Indicator	Sample 1	Sample 2
Initial moisture content, %	31.6 ± 0.2	28.5 ± 0.1
Final moisture content (storage), %	16.1 ± 0.1	17.0 ± 0.2
Acidity, degrees	27.8 ± 0.3	35.0 ± 0.3
Number of yeast cells, CFU /g	9.90×10^6	3.25×10^6
Activity of lactic acid bacteria, min	136	100

It was discovered that the acidity is high, the number of yeast cells is insufficient for effective initiation of alcoholic fermentation in the dough, and the activity of lactic acid bacteria is reduced. Obviously, this is due to the characteristics of the microbiome that cause fermentation of grape must, as well as the composition and characteristics of the nutrient medium, which in these studies is based on corn flour.

To adapt the fermentation microbiota to the conditions of bread semi-finished products, its reproduction can be carried out using nutrient media of a different composition. In this direction, studies were conducted [30] where the nutrient medium was beet molasses, which complies with DSTU 3696-98, with sucrose and malt must, in which maltose is the main carbohydrate. The aim of the research was to establish the effect of the composition of these media, the concentration of sucrose and maltose on the reproduction process of yeast: bakery, beer, alcohol and wine. For the study, a solution of molasses and malt wort of four concentrations in the range of 9–20 wt.% was prepared. The initial concentration of yeast in all cases was $1.5 \times 10^6 \pm 0.2 \times 10^6$ cells per cm^3. Yeast cultivation was carried out during the day at a temperature of 25°C under the conditions of simple batch culture. Analyzing the results of the study showed that during cultivation in a nutrient medium of beet molasses with a sucrose concentration of 9%, yeast strains behave practically the same; however, in malt must with maltose there is no such pattern. At the same time, increasing the sucrose concentration to 12% did not affect the reproduction of wine yeast. Increasing the maltose concentration to 12% in the nutrient medium also caused a decrease in the activity of yeast reproduction of all strains.

Differences in yeast biomass accumulation were established, a positive effect on the reproduction of wine and baker's yeast was found in a nutrient medium containing sucrose, and in baker's and alcohol yeast in a medium containing maltose. These results can be used to enrich the nutrient medium, improve the process of wine yeast reproduction and adapt it to the conditions of bread semi-finished products.

The issue of optimizing the composition of the nutrient medium for the propagation of fermentation microbiota, modifying fermentation systems without deteriorating their functional and technological characteristics, and searching for innovative ingredients is also attracting the attention of foreign researchers.

Byproducts of the food industry are of interest. Agave pulp is one of the most common byproducts of agave processing, formed mainly during the production of tequila and mezcal in Mexico. Its share is about 40% of the total mass of the plant. In terms of chemical composition, the pulp is characterized by a high content of structural polysaccharides: cellulose (≈ 43%), hemicellulose (≈ 19%) and lignin (≈ 15%), which determines its potential as a source of dietary fiber. At the same time, increased moisture content (60–75%) limits the microbiological stability of the ingredient, reduces its shelf life and complicates logistical use, which is one of the reasons for the insufficient utilization of this resource.

The aim of this study was to evaluate the effect of adding agave pulp and *Lactococcus lactis* NRRL B-50307 during the development of sourdough intended for the production of pastries.

During the fermentation process, an increase in proteolytic activity was discovered, which indicates an effective metabolic adaptation of the starter microbiota to the fibrous substrate of agave pulp. The intensification of enzymatic processes indicates the involvement of structural components of the pulp in biotransformation, which potentially contributes to the increase in the nutritional and functional value of the final product. It is important that the addition of agave pulp did not cause a negative impact on the rheological parameters of the dough, particularly its consistency and volume during kneading.

Evaluation of the effect of drying demonstrated that the use of moderate temperature regimes minimally affected the content of bioactive compounds and antioxidant potential of the sourdough. Although an initial decrease in the viability of microorganisms after dehydration was recorded, subsequent reactivation ensured effective restoration of the metabolic activity of the culture. This confirms the functional stability of the microbiota and its ability to reverse adaptation after dehydration.

Such techniques can be considered as a promising approach to creating starter cultures with increased functional value. The use of drying and subsequent reactivation technologies opens up opportunities for optimizing starter culture storage, reducing production costs, and expanding the use of plant byproducts in the production of environmentally friendly bakery products while maintaining the stability of fermentation processes and forming high-quality [31].

The next stage of these studies was the development of a technology for reactivating dry wine yeast in order to adapt it to the conditions of flour systems, intensify the

fermentation of dough carbohydrates, and determine the optimal fermentation parameters considering the physiological and biochemical characteristics of the culture.

4.5 Adaptation of liquid wine yeast to flour systems: production technology, fermentation parameters and impact on the quality of semi-finished products

The growing demand for products with a strong regional identity has led to increased interest in spontaneous fermentation as a source of autochthonous strains adapted to specific environmental conditions and substrate composition. The high adaptability of such cultures to fluctuations in the nutrient environment is an important prerequisite for their potential application in breadmaking.

If proper sanitary and hygienic requirements are observed, bacterial contamination of the must is insignificant; however, individual representatives of the genera *Lactobacillus* and *Fructobacillus* may participate in related biochemical processes.

For bread-making, yeasts of the genus *Saccharomyces* are technologically significant, particularly *S. cerevisiae*, *S. vini*, *S. uvarum*, *S. carlsbergensis*, *S. chevalieri*, *S. oviformis*, *S. chodati*. Their functional role in dough is fundamentally different from the conditions of winemaking and consists of ensuring alcoholic fermentation with the formation of ethanol and carbon dioxide, forming the volume of the loaves, the structure and porosity of the crumb, the synthesis of aromatic metabolites and increasing the bioavailability of bread components.

In flour medium, yeasts are subjected to osmotic and ionic stress due to low dough moisture and the presence of sodium chloride, and also function with a limited amount of simple sugars, using mainly maltose as the main source of carbohydrates. It has been established that wine yeasts are able to ferment glucose, fructose, sucrose and maltose, but the intensity of metabolism depends on their concentration, the ratio of sugars and the availability of nitrogen sources. The growth rate of cells is determined by the composition of the nutrient medium; the duration of the lag phase can be 4–8 hours depending on the cultivation conditions.

Thus, the enzymatic potential of grape must microbiota, particularly the ability to effectively utilize glucose, sucrose and maltose, indicates the possibility of its use to ferment bread semi-finished products. Taking into account the physiological and biochemical characteristics of wine yeast, the peculiarities of bread baking and the available experimental experience, it is advisable to introduce them into liquid semi-finished products as a way to ensure stable activation and increase fermentation activity in dough systems.

From the standpoint of modern food biotechnology, it is promising to form standardized methodological approaches to using wine yeast in baking according to a model close to the technology of liquid yeast with 85–90% moisture content. The implementation of such a scheme contributes to the gradual adaptation of the microbial culture to the starch-containing flour environment, minimizing metabolic stress and stabilizing the indicators of fermentation activity in the process of maintaining the semi-finished product.

Preparation of a liquid semi-finished product based on wine yeast was carried out according to a step-by-step algorithm. During the first stage, a saccharized flour brew was prepared, which served as a source of available carbohydrates and provided optimal conditions for the primary reactivation of cells. Partial hydrothermal destruction of starch with subsequent saccharification contributed to the accumulation of fermented sugars necessary for the intensification of the initial stages of culture growth.

During the second stage, dry wine yeast was reactivated on a prepared substrate with subsequent systematic (daily) feeding with a water-flour mixture with the specified moisture content. This regime ensured a gradual increase in biomass, the formation of a stable population structure and the leveling of acid accumulation and gas-forming ability. Regular renewal of the nutrient medium maintained the cells in an active physiological state and contributed to the selection of adapted forms with increased enzymatic potential.

To objectify the obtained results, control samples of liquid wine yeast were additionally prepared in a traditional water-flour system without the saccharification stage. Comparative analysis allowed to assess the effect of preliminary substrate preparation on the fermentation kinetics, the titrated acidity level and the lifting force of semi-finished products.

It was determined that a similar technological process is used in some households in the southern region of Ukraine, which indicates its empirically formed effectiveness. Scientific substantiation and unification of the specified approach create the prerequisites for the implementation of a reproducible technology for the use of wine yeast in bread-making systems with predicted functional and technological properties.

The nutrient medium was formed based on first grade wheat flour, which is characterized by a more complete chemical composition and a higher content of biologically active components. Given the prevalence of high-grade flour on the Ukrainian market, experimental samples were prepared in parallel with its use to assess the effect of flour variety on the course of activation. At this stage, dry wine yeast obtained from red grape varieties was used.

During the dilution (activation) cycle, the moisture content of the nutrient medium was monitored after the initial mixing and after each daily renewal, which was

carried out at an interval of 24 hours. The dynamics of activation on the first day were assessed at an interval of 6 hours, then once a day. Monitoring included the determination of sensory properties, lifting force as an integral criterion of the rate of CO_2 accumulation due to the metabolic activity of yeast, as well as titrated acidity, which characterizes the total content of organic acids and dissolved carbon dioxide.

The criteria for completing the activation cycle and readiness of liquid wine yeast for use in dough systems were considered to be: an increase in the volume of the semi-finished product, the formation of a pronounced fermentation aroma, an increase in acidity and the achievement of a lifting force at the level of 20–25 minutes. The humidity of the semi-finished products after mixing was 85–90%, maturation was carried out at a temperature of 27–29°C, the content of dry wine yeast was 7% to the weight of flour.

The proposed activation process creates the prerequisites for increasing the stability of fermentation processes in bread semi-finished products (**Fig. 4.1**) and ensures the adaptation of wine yeast to the specific conditions of the flour medium.

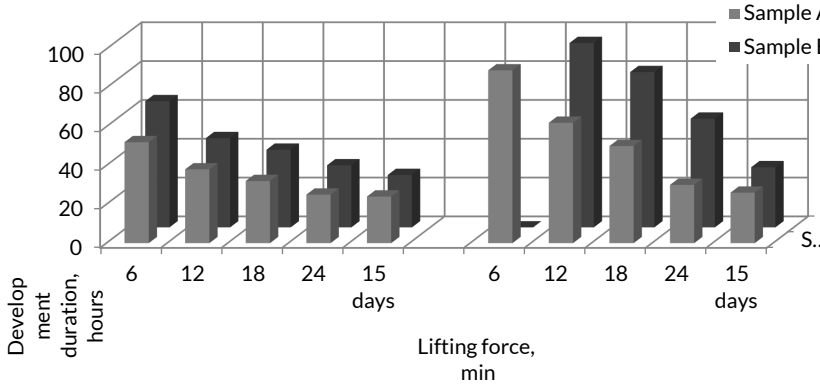

Fig. 4.1 Dynamics of lifting force during the development and maintenance of liquid wine yeast (15 days) using first and high-grade wheat flour

Analysis of experimental data shows that wheat spontaneous semi-finished products based on wine yeast achieve the technologically required fermentation activity within 24 hours (one dilution cycle) provided that saccharized flour brew from first grade flour is used as a nutrient medium. When using higher-grade flour, the time of forming sufficient lifting force is slightly increased. The development of liquid wine yeast in a traditional water-flour mixture requires 24–36 hours for first grade flour and up to 48 hours for higher-grade. At the same time, prolonged culture

maintenance for 15 days was not accompanied by a decrease in lifting force, which indicates the stability of the fermentation microbiota under the selected conditions.

The dynamics of titrated acidity, as an integral indicator of the activity of lactic acid bacteria (LAB), demonstrates more intense acid formation on the first day in samples on first grade flour, especially when using saccharized brew. This is probably due to the enrichment of the medium with available carbohydrates and biologically active substances (amino acids, vitamins), which are formed during saccharification and contribute to the rapid activation of acid-forming microflora.

In samples on a water-flour mixture from high grade flour, the increase in acidity occurred more slowly, which can be attributed to the chemical composition less saturated with biologically active components and the specificity of the microbiological profile of such flour. An increase in titrated acidity was observed during the first 6–8 days. At the same time, on the 5–6th day, the indicators of samples obtained on a water-flour mixture approached the values for systems with saccharized brew; after the 6th day, the process stabilized with the formation of an acidity level of 8.6–9.2° for first grade flour and 6.8–7.5° for high grade flour (**Fig. 4.2**).

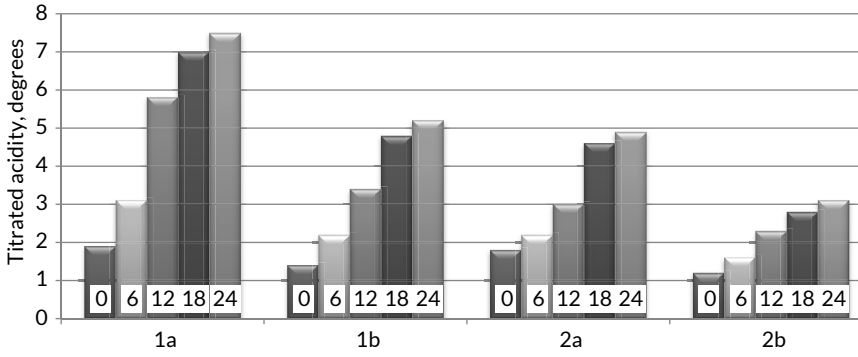

Fig. 4.2 Acid accumulation in the process of removing liquid "wine" yeast during the first 6, 12, 18 and 24 hours using as a nutrient medium a saccharized brew (1) and a water-flour mixture (2) based on flour of the first (*a*) and high (*b*) grades

Lactic acid fermentation is an important stage in the development of spontaneous sourdough starter cultures and liquid wine yeasts (**Fig. 4.3**). The technological role of lactic and related organic acids is to regulate the acidity of the medium, initiate positive structural and functional changes in flour biopolymers, create favorable conditions for the development of yeast and at the same time inhibit undesirable microbiota, particularly putrefactive bacteria and representatives of the

genus *Leuconostoc*, which are associated with microbiological spoilage. The combination of these effects determines the stability and technological reliability of the resulting semi-finished products in bakery systems.

The development stage of wheat spontaneous liquid "wine" yeast (sourdough) is aimed at the initiation and selective activation of representatives of the genus *Saccharomyces*, introduced with dry wine yeast, as well as the accumulation of functionally active fermentation microbiota, including lactic acid bacteria.

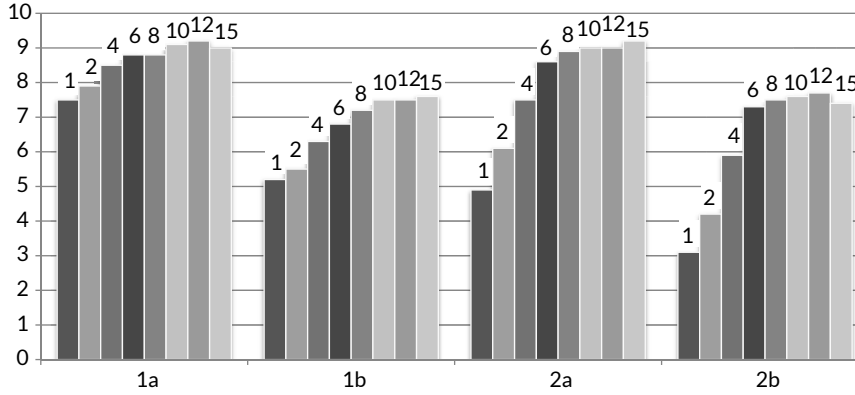

Fig. 4.3 Changes in titrated acidity during the process of maintaining liquid "wine" yeast during the first 15 days using as a nutrient medium a water-flour mixture based on flour of the first (*a*) and high (*b*) grades for samples on saccharized brew (1) and water-flour mixture (2)

The combined metabolic activity of this consortium provides intensive gas formation, synthesis of organic acids, aromatic and flavor-forming compounds and other technologically significant metabolites. At the same time, physical, chemical, colloidal and enzymatic transformations of flour biopolymers occur, which determine the structural and mechanical properties of the dough and the quality of the finished product.

It has been established that when using saccharized flour brew as a nutrient medium, liquid "wine" yeast reaches the required level of fermentation activity at the end of the first day of fermentation, which indicates effective adaptation of the culture to the carbohydrate composition of the system.

At the next stage of the research, the technological properties of liquid "wine" yeast o btained from white and red grapes were evaluated when they were added to a saccharized brew in an amount of 5 and 7% to the weight of first grade wheat flour. Given the significant differences between the conditions for fermentation of

grape must and fermentation of bakery semi-finished products, as well as taking into account the production risks associated with possible power supply interruptions, the influence of temperature regimes (19–22°C and 25–27°C) on the formation of bakery properties of these cultures was additionally investigated. The generalized results of the experiments are presented in **Table 4.4**.

According to the obtained experimental data, it was established that the highest indicators of the baking value of liquid "wine" yeast are achieved when they are added in an amount of 7% to the weight of wheat flour (first grade) using a culture isolated from grapes, at a fermentation temperature of 25–27°C. Under these conditions, maximum fermentation activity, optimal indicators of lifting force, and stable acid-accumulating ability of the semi-finished product were observed.

A thorough description of sensory properties is necessary to identify the technological readiness of the semi-finished product, prevent the development of undesirable microflora and ensure the reproducibility of results when using wine yeast. This is especially important when using non-traditional yeast cultures and alternative starters, where there are no established regulatory indicators. In order to control the processes of yeast activation and maturation during the first day every 6 hours, and then daily, the sensory properties (**Table 4.5**) of a liquid semi-finished product based on wine yeast were evaluated.

Sensory evaluation showed the presence of intense visual signs of fermentation (gas formation, volume increase), a formed aromatic profile with the dominance of metabolites of alcoholic and lactic fermentation. Sourdoughs based on wine yeast from red grapes were characterized by a more pronounced tone of alcoholic fermentation products with light grape notes and a darker shade of color, which is due to the characteristics of the starting material and the spectrum of secondary metabolites.

Table 4.4 Baking properties of liquid "wine" yeast

Indicators	Wine yeast from white grapes				Wine yeast from red grapes			
	5% to the weight of flour		7% to the weight of flour		5% to the weight of flour		7% to the weight of flour	
Variant	1	2	3	4	5	6	7	8
Ripening temperature, °C	19–22	25–27	19–22	25–27	19–22	25–27	19–22	25–27
Moisture content, %	90	90	90	90	90	90	90	90
Titrated acidity, degrees (after 24 hours)	6.0	7.1	6.6	7.8	5.8	6.4	6.9	7.5
Lifting force, min. (after 24 hours)	68	29	56	25	80	50	75	32

Table 4.5 Sensory properties of liquid semi-finished product based on wine yeast

No.	Quality indicators	Sensory properties
1	Appearance	Porous, viscous
	Surface	Foam on the surface
	Color	Light gray with a faint burgundy tint; light cream; gray-pink/red (depending on the grape color in the must)
2	Scent	The smell is clean, sour-bread, fruity with a slight alcohol note. Typical for a bread semi-finished product
3	Consistence	Thick, with gas bubbles inside

Reducing the fermentation temperature to 19–22°C or reducing the dose of dry "wine" yeast was accompanied by a prolonged stage of removal and maturation of the liquid semi-finished product, which indicates a decrease in the intensity of metabolic processes and requires further optimization of cultivation modes.

In order to determine a rational technological scheme for dough preparation, comparative studies were conducted using straight dough, thick leaven and liquid leaven technologies. Samples made using the traditional yeast bread technology using pressed yeast were used as a control.

Wheat bread products were produced using liquid and thick leavens and straight dough in order to establish its effect on the formation of consumer and technological characteristics of the product. The dough was prepared using thick leaven (47–50% moisture) and liquid leaven (68–70% moisture) with 50 and 30% flour respectively. The dough was kneaded manually, then placed for fermentation in a thermostat at a temperature of 27–29°C, the total duration of fermentation was 180–240 minutes. After the dough was fermented, the dough was kneaded with the addition of the remaining flour and salt. Kneading was carried out under laboratory conditions in a farinograph mixer. It was placed for fermentation for 60–90 minutes, every 30 minutes after the start of fermentation it was kneaded by hand. During fermentation, the dough increased in volume and had a convex shape. The dough was also prepared in a straight way, where all the ingredients according to the recipe, including salt in the form of a solution, were mixed in a farinograph mixer until a dough with the necessary structural and mechanical properties was formed. The dough moisture content in all samples ranged from 43.6 to 44.2%.

In the control samples, which were prepared according to traditional technological recommendations, the dosage of pressed yeast was 1% to the weight of flour for leavened dough, and 3% for straight dough.

The readiness of the dough was assessed by achieving the normative titrated acidity and lifting force indicators and by the set of sensory characteristics, in

accordance with the current technological regulations. Taking into account the results obtained, the parameters of the technological process were justified (**Table 4.6**) and a comparative assessment of the quality of semi-finished products (leaven and dough) was carried out.

Table 4.6 Parameters of the technological process for making leaven and dough

Indicators	Control			From liquid "wine" yeast		
	liquid leaven	thick leaven	straight dough	liquid leaven	thick leaven	straight dough
Leaven preparation						
Moisture content, %	70	50	–	68	47	–
Fermentation time, min	240	240	–	300	240	–
Lifting force, min	24	21	–	18	21	–
Final acidity, degrees	5.0	3.5	–	5.5	5.9	–
Dough preparation						
Moisture content, %	44.0	44.2	43.6	44.1	43.8	43.5
Fermentation time, min	60	60	180	90	90	240
Lifting force, min	16	14	13	7	11	18
Final acidity, degrees	3.8	3.5	3.1	3.9	4.3	4.2

After the fermentation stage, the dough was divided into loaves, shaped and sent to proof at a temperature of 34–36°C. In samples with "wine" yeast and though prepared with straight dough, the duration of proofing was within 60–90 minutes, with pressed yeast was 45–60 minutes. After the dough loaves increased in volume, they were moved to the baking chamber for baking. The baking duration at a temperature of 180–220°C for all samples was almost the same.

4.6 Sensory, physical and chemical quality assessment of bread products made by different dough preparation methods

Sensory, physical and chemical assessment of the quality of wheat bread products made with liquid and thick leavens and by the straight dough method was carried out in order to establish its effect on the formation of consumer and technological characteristics of the products. The research was carried out according to unified methods using standard instrumental and sensory approaches. Sensory properties were assessed according to a descriptive scale taking into

account the shape, surface condition, crust color, crumb structure, aroma and taste. Physical and chemical parameters included moisture content, acidity, specific volume, porosity, shape stability and texture indicators. Comparative analysis was carried out 4–24 hours after baking in order to take into account the stabilization of the crumb structure.

Bread products made with liquid leaven were characterized by a more developed porous structure with thin-walled pores of predominantly rounded shape. The crumb had increased elasticity and uniform texture, which indicated intensive gas-forming processes at the fermentation stage. The crust was characterized by a uniform golden-brown color and a pronounced aroma, formed as a result of active fermentation and Maillard reactions. The products were characterized by a balanced taste with moderate acidity and without noticeable foreign flavors. During sensory evaluation, the samples prepared using liquid dough demonstrated a somewhat higher aromatic intensity compared with the other variants.

Bread produced with thick leaven showed a denser crumb with a finer internal structure. At the same time, the distribution of pores was less uniform. This feature can likely be explained by the lower hydration level of the finished product as well as by differences in the course of fermentation processes.

In addition, these samples formed a thicker crust with a more intense coloration, which may be related to the longer fermentation period and the associated biochemical transformations in the dough. The taste of such bread was perceived as richer, with more pronounced acidity and a more expressed enzymatic character.

Overall, the sensory characteristics suggest that the use of thick leaven contributes to the formation of a flavor profile closer to that traditionally associated with artisanal bread-making technologies.

A visual image of a cross-section of finished bread products prepared with pressed yeast is shown in **Fig. 4.4**, and with liquid wine yeast in **Fig. 4.5**.

Fig. 4.4 Cross-sectional view of bread products made from wheat flour with pressed yeast using different dough preparation methods: 1 – without leaven; 2 – with liquid leaven; 3 – with thick leaven

Fig. 4.5 Cross-sectional view of bread products made from wheat flour made with wine yeast using different dough preparation methods: 1 – without leaven; 2 – with liquid leaven; 3 – with thick leaven

Physical and chemical analysis (**Table 4.7**) showed that crumb moisture content in products made with liquid leaven was statistically higher compared to analogues made with thick leaven. The specific volume of bread made with liquid leaven exceeded the corresponding indicator in samples made with thick leaven, which correlated with the greater gas-holding capacity of the dough. Titrated acidity was higher in products made with thick leaven, which confirms the more intensive accumulation of organic acids during the fermentation process. The shape stability of products made with thick leaven was characterized by increased values, which is associated with a stronger gluten framework structure. Porosity and structural and mechanical properties were consistent with the results of sensory evaluation.

Table 4.7 Physical and chemical qualities of wheat bread made with pressed yeast (control) and with spontaneous starters from wine yeast

Indicators	Control			With sourdough		
	liquid leaven	thick leaven	straight dough	liquid leaven	thick leaven	straight dough
Moisture content, %	43.2	43.3	42.9	43.0	42.8	42.7
Acidity, degrees	2.9	2.7	2.2	3.0	3.2	3.1
Porosity, %	73	72	69	73	71	68
Specific volume, $cm^3/100$ g	3.17	3.06	2.95	3.20	3.09	2.85
Shape stability, H/D	0.58	0.56	0.56	0.50	0.49	0.47

The obtained data indicates that the method of dough preparation is a significant factor in shaping the quality of wheat bread products. A liquid leaven

provides intensification of gas formation and the formation of a more developed porous structure. The porosity of bread is a decisive factor affecting its texture. A thick leaven contributes to the accumulation of acidity and the enhancement of the taste and aroma profile. The choice of a technological process allows to purposefully regulate the structural and sensory characteristics of the finished product.

The best sensory properties and physical and chemical indicators were characterized by products made with liquid leaven using liquid "wine" yeast. In terms of porosity and specific volume, bread made on semi-finished products of spontaneous fermentation was not inferior to the control sample made with traditional liquid leaven. Studies have established a significant improvement in the elasticity of the crumb of bread samples made on liquid and thick leavens with the addition of wine yeast compared to the control samples. However, an increase in the titrated acidity of the products by 0.6–0.9 degrees was established, and it slightly exceeds the normalized values for traditional bread made from premium wheat flour. This should be adjusted in the standards for bread products made using the technology under study. From the standpoint of artisanal bread baking, a differentiated approach to the use of leavens depending on the desired quality indicators is advisable. Thus, optimization of fermentation parameters opens up opportunities for increasing the stability and predictability of bread quality under different production models. It was found that the staling process of bread samples made with wine yeast sourdough proceeded more slowly than in control samples. Slower rates of freshness loss in samples with sourdough were also established by the indicator of elastic deformation of the bread crumb and by changes in its crumbliness.

Thus, the effectiveness of using wheat spontaneous sourdough with wine yeast has been established, as an example of ancient national traditions of dough preparation, which have been preserved in Ukrainian villages of southern Ukraine. It is promising as a comprehensive solution to bread baking problems related to improving quality and expanding the range of products.

At the same time, it was found that in order to revive the ancient national traditions of baking, their effective introduction and practical implementation at domestic enterprises, it is necessary to clarify and formulate the theoretical foundations of bread technologies using spontaneous sourdough, develop an informative base and practical recommendations for bread producers, which will provide for adaptation to local raw materials, common production models and equipment in the conditions of industrial bakeries and small, artisanal and craft bakeries. This requires combining the efforts of bakers with specialists in the fields of

history, ethnography, agriculture, chemistry, biochemistry, microbiology, nutrition, restaurant management and others. Only under such conditions is it possible to realize the high potential of sourdough in solving the problems of the industry and the prospects for expanding the range of products that can be presented on the market, including under the popular lines "according to ancient, ethnic, authentic technologies", "living", "artisanal" bread, "for health", "with improved physiological properties".

4.7 Conclusion

A review of current data on the evolution of bread in the different regions of the world, an analysis of ancient and modern traditions of bread making in terms of choosing a development strategy for modern domestic enterprises of the bread and restaurant business, which is aimed at improving technologies and the range of bakery products, solving industry problems and improving product quality, was conducted. The work substantiates the high potential of wheat spontaneous starters authentic for Ukraine on wine yeast in solving bread baking problems and the feasibility of studying their technologies and properties.

Experimental studies have established that the parameters of sourdough preparation and their properties are significantly influenced by the recipe, chemical, and microbiological composition of the ingredients. It has been proven that due to the use of dried wine yeast and saccharized brew in the fermentation cycle, the sourdough acquires the necessary biotechnological properties within a day.

It was established that products made on liquid wine yeast have strong sensory characteristics, specific shades of taste and aroma, crust color, pore structure. In terms of porosity and specific volume, bread made with semi-finished products of spontaneous fermentation was not inferior to the control sample made with traditional liquid leaven. Studies have established a significant improvement in the elasticity of the crumb of bread samples made with the use of liquid and thick leavens on liquid wine yeast compared to the control samples.

All this gives grounds to assert the prospects of using spontaneous sourdough starters in the bread and restaurant business to improve technology and product range, and comprehensively improve its quality. However, it is noted that there is a need to continue research to create an information and regulatory base on national traditions, recipes, and product range in terms of historical periods and different regions, to formulate the theoretical foundations of bread technology using spontaneous starters, to develop measures to form the proper quality of

products, and to adapt them to the working conditions of modern industrial and craft producers.

Conflict of interest

The authors declare no conflict of interest with respect to this paper, as well as the published research results, including financial aspects of conducting the research, obtaining and using its results, as well as any non-financial personal relationships.

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Data availability

The manuscript has no associated data.

Use of artificial intelligence statement

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

Authors' contributions

Nataliia Slobodyanyuk: Conceptualization, Formal analysis.
Oksana Tkachuk: Text writing, Information collection, Research, Analysis and systematization of results.
Tetiana Brovenko: Validation,Resources, Methodology, Research, Formal analysis.
Tetiana Lebedenko: Text writing conceptualization, Research, Validation, Formal analysis.
Halyna Tolok: Resources, Methodology.
Tamara Novichkova: Methodology, Formal analysis.

Petro Drozd: Resources, Methodology.
Mykola Gruntkovskyi: Resources, Methodology.

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CHAPTER 5

Technological and qualitative aspects of enriching wheat bread with oyster mushroom paste

Oleksandr Sokot

Abstract

The article investigates the feasibility of using oyster mushrooms (*Pleurotus ostreatus*) in the form of paste as a functional ingredient in wheat bread produced according to a traditional formulation. The use of by-products from primary mushroom processing (stems and cluster bases) is substantiated as a way to reduce raw material costs and improve the economic efficiency of bread production. Yield coefficients of semi-finished mushroom products obtained by boiling and drying different oyster mushroom strains were determined, and the dry matter content of various parts of fruiting bodies was analyzed. The effect of adding 10%, 20%, and 30% mushroom paste on the physicochemical properties of wheat bread, including moisture content, acidity, porosity, specific volume, baking loss, shrinkage, and crumb water absorption capacity, was evaluated. All experimental bread samples complied with the requirements of State Standard of Ukraine (DSTU 7517:2014 "Wheat Bread"). The results demonstrate that the incorporation of mushroom paste leads to partial changes in the physicochemical characteristics and amino acid profile of bread without exceeding regulatory quality limits. The findings confirm the potential of oyster mushroom paste as a promising ingredient for the development of functional bakery products.

Keywords

Bread, mushroom paste, oyster mushroom, functional foods, semi-finished product yield coefficient, technical characteristics.

5.1 Problem statement

The economic aspect is one of the keys to the success of any industry, and bread production is not an exception. The results of a scientific analysis of the Ukrainian bakery market indicate several needs [1, 2]; among them, the expansion of the

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product range is particularly noteworthy. This can be achieved, for instance, by improving the assortment in accordance with nutritionists' recommendations and consumer preferences, in response to the growing interest in healthy nutrition [3].

At the same time, researchers note the technological difficulties of such innovations, associated with a decrease in the volume of products, deterioration of organoleptic indicators, as well as additional costs during production. Despite the high scientific interest in this topic, the issue of the optimal combination of functional additives while maintaining high organoleptic indicators and economic feasibility of bread production remains insufficiently studied.

5.2 Analysis of recent research

An analysis of the European bakery market conducted by M. Sychevskyi et al. [4] identified sustained growth in demand for functional bakery products as one of the key long-term trends. Given the structural similarities and integration processes within the European and Ukrainian food markets, this trend may also be considered relevant for the further development of the bread industry in Ukraine. This tendency toward functionalization is further supported by other researchers, who emphasize that current trends in the Ukrainian bread market include growing consumer interest in healthy nutrition, increased demand for high-margin and craft bakery products, and the expansion of assortments with improved health characteristics.

In accordance with current requirements, domestic scientific research is aimed at enriching bread with plant components with a high content of dietary fiber, antioxidants, and biologically active substances. Thus, positive results were obtained in experiments with the addition of various plant-based ingredients and alternative cereal raw materials into bread formulations, including spice plants [5], flaxseed meal [6], amaranth flour [7], hemp seeds [8], asparagus waste [9] and spelt flour [10] to bread.

It should be noted that this research direction represents a global trend. Studies by international authors report numerous examples of incorporating non-traditional plant-based ingredients into bread formulations to improve its properties, including soybeans and sweet potatoes [11, 12], green tea [13], herbs and spices (coriander leaves, ginger) [14, 15], fruits and their by-products (grape seeds) [16], and green coffee beans [17].

Particular scientific interest has been focused on the incorporation of bioactive compounds derived from mushrooms into flour-based products. The addition of oyster mushroom powder to wheat flour has been shown to increase crude protein

content [18], while also enhancing the mineral composition by elevating levels of sodium, potassium, calcium, and magnesium [19].

In conclusion, an analysis of recent scientific publications indicates a growing interest in the development of functional bakery products enriched with mushrooms as a valuable source of bioactive compounds. Oyster mushrooms, which represent an accessible raw material in European and Asian countries, as well as the by-products of their primary processing, may be used as an additive in bread with enhanced functional properties.

5.3 Objectives of the study

The aim of this work was to investigate the feasibility of using the common oyster mushroom in the form of minced mass (paste) as an additive capable of improving the functional characteristics of wheat bread produced according to a conventional formulation. In order to reduce raw material costs, the study also assessed the practicality of utilizing by-products from the pre-sale sorting of fresh oyster mushrooms, including trimmed stems and cluster bases.

During the study, the following objectives were established:

1. To determine the compliance of bread samples containing 10%, 20%, and 30% paste prepared from boiled oyster mushrooms with the requirements of DSTU 7517:2014 "Wheat Flour Bread".

2. To identify changes in the physicochemical properties of mushroom-enriched bread, including moisture content, titratable acidity, porosity, water absorption capacity, baking loss, shrinkage during storage, and specific volume.

3. To investigate changes in the composition of essential amino acids in the produced samples.

5.4 Materials and methods

Experiments aimed at assessing the quality of the raw materials (mushrooms) were conducted in the laboratory of ESMASh-3 LLC (Kyiv, Ukraine). Semi-finished product yield coefficients were calculated as the ratio of the mass of the obtained product to the mass of the initial raw material.

The investigation of bread samples enriched with mushroom paste was carried out in the laboratory of the Department of Bakery and Confectionery Technology, National University of Food Technologies (Kyiv, Ukraine). The experimental design

involved the production of four variants of wheat bread made from premium-grade wheat flour with the addition of mushroom paste prepared from common oyster mushrooms of unsatisfactory market appearance (cracked caps, separated parts of fruiting bodies) as well as by-products of primary processing (cluster bases and stems). The first sample served as the control and was prepared according to a conventional formulation. The experimental variants contained 10%, 20%, and 30% mushroom paste, with a corresponding replacement of part of the water in the bread formulation by the water contained in the mushroom paste.

Mushroom paste was prepared from mushroom raw material boiled for 5 minutes and partially cooled on a metal sieve to remove excess water. The prepared mushrooms were ground using a blender to obtain a homogeneous paste.

Dough mixing for the experimental variants was performed using a dough mixer. Baking was carried out in a convection oven for 30 minutes at 200°C. The bread was cooled under ambient conditions by placing it on racks. Product quality parameters were determined in accordance with DSTU 7517:2014 "Wheat Flour Bread". All experiments were conducted in triplicate.

Moisture content, specific volume, porosity (using the Zhuravlev apparatus), water absorption capacity of the bread crumb, baking loss, and shrinkage coefficients were determined according to standard methods.

Bread acidity was determined in accordance with State Standard of Ukraine (DSTU 7045:2009 "Bakery Products. Methods for Determining Physicochemical Parameters", including amendments). Amino acid composition was analyzed using standardized methods at the Experimental and Biological Center of the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine.

Statistical analysis was performed using Microsoft Office Excel 2016 MSO (16.0.4266.1001). The obtained data were analyzed using both one-way ANOVA and two-way ANOVA with replication via the QI Macros 2020 add-in for Excel 2016. Mean values were compared using Duncan's multiple range test. Differences were considered statistically significant at $p < 0.05$. All experiments were conducted in triplicate.

5.5 Discussion of results

The nutritional and technological value of mushroom raw materials is primarily determined by their dry matter content; therefore, it is advisable to analyze the yield of semi-finished products not only after boiling followed by grinding into a paste, but also after complete moisture removal through drying. Such an approach allows

for an objective assessment of raw material losses associated with its structural and chemical characteristics and enables a more accurate interpretation of the results obtained from the use of paste-like semi-finished products in wheat bread technology.

The yield coefficients of semi-finished products (Y_{SP}) of four strains of the common oyster mushroom were compared using two raw material processing methods: boiling and drying (**Fig. 5.1**).

The lowest losses (2.6%) were observed after boiling mushrooms of Strain 433 (Y_{SP} = 0.9737 ± 0.0219), whereas for the other strains this parameter was close to 10%: Strain 16 – 0.9018 ± 0.0424; Strain 62 – 0.8997 ± 0.0597; Strain 1004 – 0.8973 ± 0.0203 (the highest losses). However, no statistically significant differences in Y_{SP} after boiling were found among the tested strains.

A significantly higher Y_{SP} after drying ($p < 0.05$) was determined for raw material obtained from oyster mushroom Strain 433 (0.1149 ± 0.0055), whereas Strain 16 demonstrated the lowest value (0.0910 ± 0.0045). That is, Strain 433 had the lowest raw material losses (88.5%), while Strain 16 had the highest (90.9%), with a difference of 2.4%.

The lower mass losses during boiling observed for Strain 433 may be attributed to its higher dry matter content.

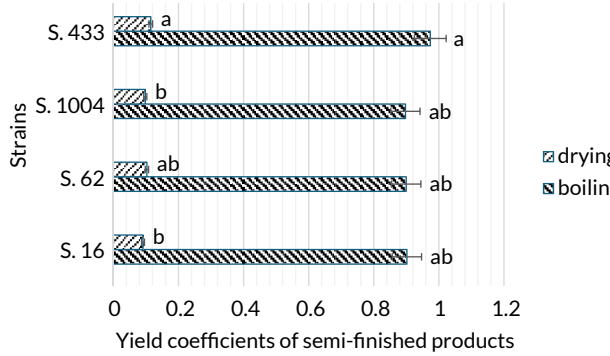

Fig. 5.1 Yield coefficient of semi-finished products after boiling and drying of Pleurotus ostreatus (Strains 16, 62, 433, and 1004). Statistically significant differences between the results ($p < 0.05$) are indicated by different letters of the Latin alphabet

A comparison of dry matter (DM) content in the fruiting bodies, caps, and stems with trimmed cluster bases of the aforementioned strains demonstrated the consistency of this parameter for raw material obtained from stems and cluster base trimmings (**Fig. 5.2**).

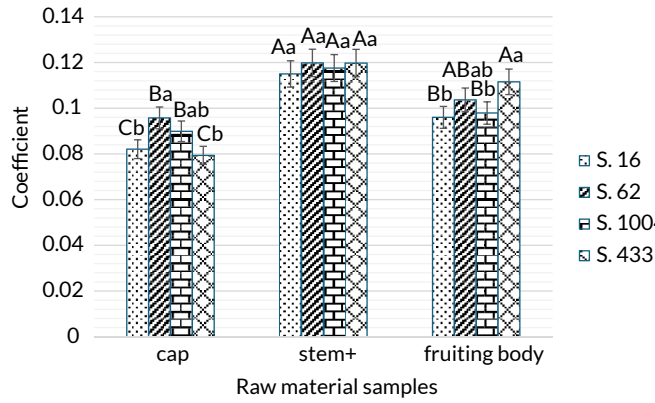

Fig. 5.2 Dry matter content in different raw material types obtained from Pleurotus ostreatus Strains 16, 62, 433, and 1004. Statistically significant differences between the results ($p < 0.05$) are indicated by different letters of the Latin alphabet: factor A (raw material type) is denoted by uppercase letters, and factor B (strain) by lowercase letters
*Note: *stem+ — designation for the mixture of stem waste and trimmings of cluster bases*

The highest Y_{SP} were observed when stems and cluster bases of Strains 433 and 62 were used (0.1198 ± 0.0022 and 0.1198 ± 0.0034, respectively). However, no statistically significant differences ($p > 0.05$) were found compared with Strains 16 and 1004, which showed Y_{SP} values of 0.1150 ± 0.0018 and 0.1176 ± 0.0025, respectively.

According to the results of the two-way ANOVA with replication, a statistically significant difference between the means was found for raw material types (factor B; i.e., caps, stems + trimmings, and whole fruiting bodies), whereas no significant differences were detected among the strains (factor A). However, the calculated mean dry matter (DM) content of caps and whole fruiting bodies showed greater variability compared with stems + trimmings when analyzed by strain (factor A). Specifically, the highest DM content was observed in the caps of Strain 62 (0.0958 ± 0.0002), whereas the lowest value was recorded for Strain 433 (0.0794 ± 0.0009), corresponding to a difference of 1.91%.

Whole fruiting bodies of Strain 433 were characterized by a significantly higher DM content (0.1116 ± 0.0087) compared with Strains 16 (lowest value, 0.0960 ± 0.0011) and 1004 (0.0979 ± 0.0034). In contrast, Strain 62 did not differ significantly from the other experimental results in this parameter (0.1037 ± 0.0021). Thus, the smallest difference in mean dry matter content between caps and stems + trimmings was 1.93%, whereas the largest reached 4.4%. These findings

support the feasibility of using stems and cluster base trimmings as raw material for mushroom paste production. The technological advantages of this approach include: (1) reduced dependence of the process on raw material variability; (2) production of a semi-finished product with higher dry matter content; and (3) reduced raw material costs, since stems and trimmings constitute by-products of cleaning and sorting prior to fresh sale or processing into canned products. The obtained results are consistent with previous publications investigating the processing of other *Pleurotus ostreatus* strains, confirming the stability of the technological parameters of boiling and drying processes across different strains of this species [20, 21].

The technological quality parameters of bread samples containing different levels of mushroom paste were evaluated. According to DSTU 7517:2014, the maximum moisture content of wheat bread may range from 45% to 50%, depending on the product category. The obtained results indicate a significant increase in relative moisture content in the variants enriched with mushroom paste compared with the control sample (**Fig. 5.3**).

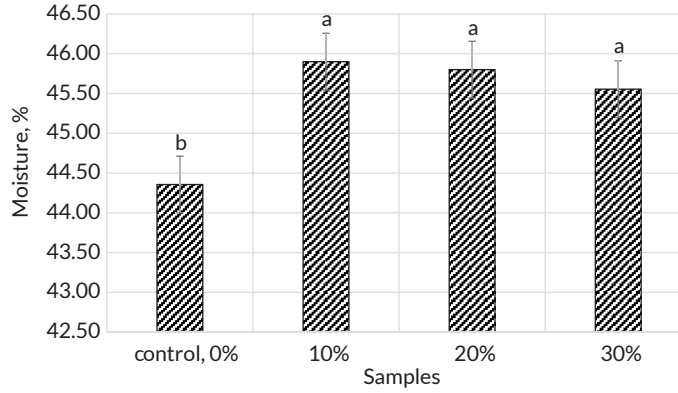

Fig. 5.3 Moisture content of bread with different levels of mushroom paste

The highest moisture content was observed in samples containing 10% mushroom addition (45.90 ± 3.36%), while the control samples exhibited 44.36 ± 2.22%. As the content of mushroom raw materials increased, the relative humidity of the product decreased, although no statistically significant difference between the experimental variants was found. This fact may be related to the presence of fungal polysaccharides, which are characterized by their ability to bind moisture with heat-resistant protein-glycan complexes, which, according to previous studies, have

a high moisture retention coefficient. Nevertheless, all experimental variants remained within the permissible moisture limits defined by the standard, which specifies a maximum of 46% for pan bread made from premium wheat flour.

Statistical analysis revealed an inverse linear correlation between baking yield and the increasing proportion of mushroom raw material (**Fig. 5.4**).

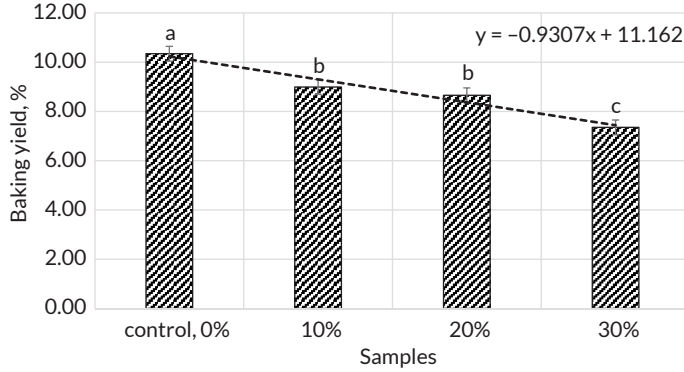

Fig. 5.4 Baking yield of bread with varying mushroom paste content

The highest baking yield (10.34 ± 1.76%) was observed in the control samples, while the lowest (7.35 ± 0.25%) was found in samples containing 30% mushroom paste, supporting the hypothesis of water retention by complex compounds formed between mushroom and flour components.

No clear trends were identified in the dynamics of the bread shrinkage coefficient; significant differences were observed only for samples with 10% mushroom addition (5.92 ± 0.47), whereas the other samples did not differ significantly from each other (**Fig. 5.5**). The results of the conducted experiments do not allow a definitive explanation of the observed effect, indicating that further studies are required.

All investigated variants complied with the requirements of the national standard DSTU 7517:2014 regarding porosity (not less than 70%). The highest porosity was observed in the samples containing 20% mushroom addition (77.34 ± 1.33%), whereas the lowest value was recorded for bread with 30% mushroom addition (**Fig. 5.6**).

No clear trends were identified in the changes of this parameter with increasing mushroom content. The observed results may have been influenced by technological aspects of sample preparation and specific testing conditions.

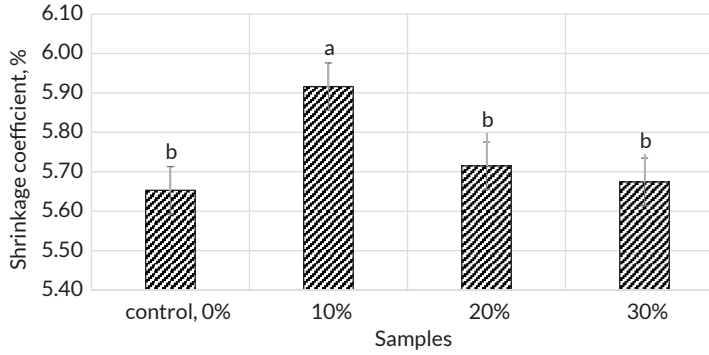

Fig. 5.5 Shrinkage of bread with varying mushroom paste content

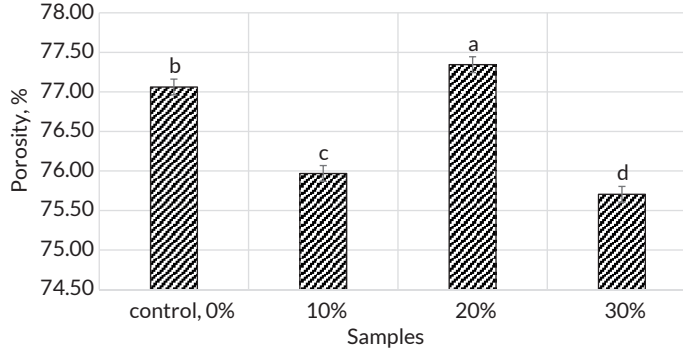

Fig. 5.6 Porosity of bread with varying mushroom paste content

No significant differences in specific volume were found between the control sample (262.7 cm^3/100 g) and the sample containing 10% mushroom paste (262.9 cm^3/100 g). In contrast, the other variants exhibited a substantial decrease in this parameter, reaching 209.7 cm^3/100 g in the sample with 30% mushroom paste, which represented the lowest value recorded in the study (**Fig. 5.7**).

Accordingly, an overall decreasing trend in specific volume was observed with increasing proportions of mushroom raw material in the bread formulation. Thus, additional incorporation of mushroom paste may adversely affect the overall appearance of the products.

Comparison of titratable acidity among the experimental variants demonstrated that the addition of mushroom paste to the dough had a noticeable effect on this parameter. However, the control sample and the variant containing 10% mushroom

paste exhibited identical acidity values (1.2°). Therefore, at this concentration, the mushroom ingredient did not affect bread acidity. Increasing the proportion of mushroom paste to 20% and 30% resulted in an increase in acidity to 1.3°, i.e., by 0.1 units (**Fig. 5.8**).

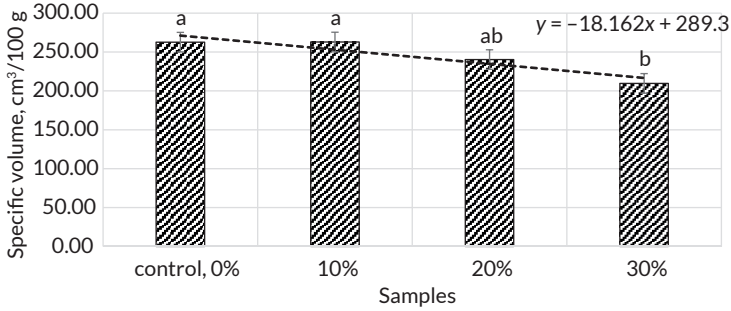

Fig. 5.7 Specific volume of bread with varying mushroom paste content

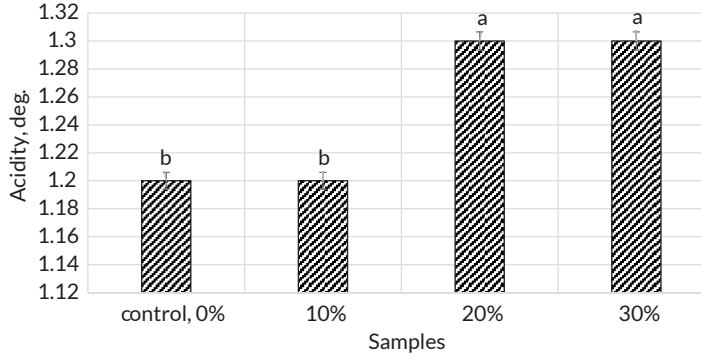

Fig. 5.8 Titratable acidity of bread with varying mushroom paste content

Despite the slight increase, the acidity of all obtained samples remained relatively low and fully complied with the requirements of DSTU 7517:2014, which specifies a maximum permissible value of 3.5°.

The water absorption capacity of the crumb was evaluated, revealing a significant increase with the addition of 10% mushroom paste, whereas increasing the mushroom content to 20% and 30% resulted in a decrease in this parameter (**Fig. 5.9**).

The lowest value was recorded in the sample containing 30% mushroom paste. This finding may be explained by the increased content of hydrophilic mushroom

polysaccharides, which retain water even after baking. However, at higher inclusion levels, they contribute to an increase in the overall moisture content of the product (**Fig. 5.3**), which in turn reduces the water absorption capacity of the bread.

Analysis of the amino acid composition of bread samples with varying mushroom paste content did not reveal a consistent trend in the levels of essential amino acids compared to the control sample (**Fig. 5.10**).

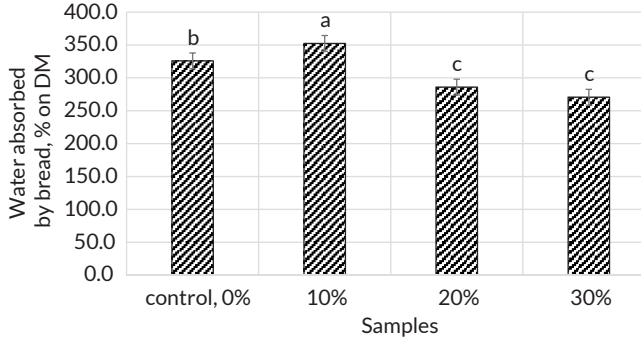

Fig. 5.9 Water absorption coefficient of bread with varying mushroom paste content

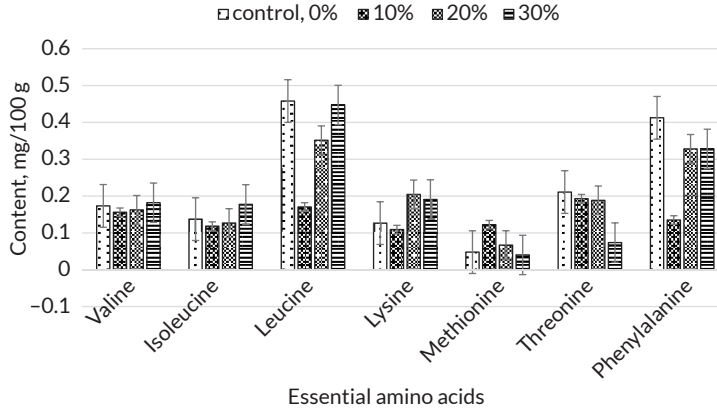

Fig. 5.10 Results of essential amino acid determination in bread with varying mushroom paste content

All bread variants exhibited high levels of leucine and phenylalanine (except for the sample containing 10% mushroom paste). The lowest values among the

variants were recorded for methionine, ranging from 0.0401 mg/100 g (30%) to 0.1223 mg/100 g (10%). It was also determined that the addition of 20% and 30% mushroom paste increased the levels of isoleucine and lysine, while significantly decreasing the contents of threonine and methionine.

The obtained data do not provide a definitive conclusion regarding the effect of mushroom paste incorporation on the content of essential amino acids in bread, indicating the need for further research.

5.6 Conclusions

The results of the study demonstrate the technological stability of mushroom paste obtained from different strains of *Pleurotus ostreatus*. The use of stems and trimming residues for the production of the mushroom semi-finished product may offer certain economic advantages. The incorporation of mushroom paste at levels ranging from 10% to 30% (flour basis) does not violate the requirements of DSTU 7517:2014 for wheat bread quality; however, it partially alters the physicochemical properties of the products, which should be considered when determining storage conditions and commercialization parameters.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper, as well as the published research results, including the financial aspects of conducting the research, obtaining and using its results, as well as any non-financial personal relationships.

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Use of artificial intelligence statement

The author confirm that he did not use artificial intelligence technologies when creating the current work.

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Authors' contributions

Oleksandr Sokot: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review and editing.

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CHAPTER 6

Improvement of technology of fish pastes with the addition of non-traditional raw materials

Nataliia Holembovska
Valentyna Israelian
Natalia Slobodyanyuk
Anastasiia Ivaniuta
Inna Stetsyuk
Inna Kurbatova
Vasyl Shynkaruk
Yaroslav Rudyk

Abstract

The growing interest of consumers in functional fish products has contributed to the development of innovative formulations enriched with biologically active and nutritionally valuable components. This study investigated the effect of including goji berries in the formulations of crucian carp-based fish sticks. Three experimental samples were developed: control – without herbal additives, sample 1 – with 2% goji berries, sample 2 – with 4% goji berries and sample 3 – with 6% goji berries. The physicochemical parameters of the products were studied, in particular, moisture, protein, fat, minerals and acidity, as well as technological characteristics – moisture retention capacity, color, texture and sensory characteristics. The inclusion of berries increased the mineral content, while the moisture content of the products decreased (from 78.0 to 68.0%). The samples with berries demonstrated improved sensory properties and increased structural density, which positively affected the textural characteristics.

When stored at 4°C for 4 days, the samples with goji berries maintained the stability of physicochemical parameters: changes in acidity and peroxide value were minimal, and sensory properties were high, indicating good oxidative and microbiological stability. The organoleptic evaluation showed improved taste characteristics and aroma harmony, which were most pronounced in sample 2.

The results of the study confirm the feasibility of using plant biologically active additives in fish pastes to increase nutritional value, functional properties and

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organoleptic appeal. Further studies are recommended to assess microbiological stability during long-term storage and study consumer preferences for introduction into production.

Keywords

Crucian carp, goji berries, biologically active additives, physicochemical properties, organoleptic properties, texture, oxidative stability, nutritional value.

6.1 Introduction

Recently, the range of fish culinary products in the world has expanded significantly. These trends are echoed by domestic producers. For example, the production of canned food, salted fish, and smoked products has decreased significantly due to rather subtle undesirable changes in the product as a result of the use of strict sterilization regimes, the high content of table salt in salty and spicy products, and the presence of harmful carcinogenic substances in smoke-smoked products [1].

The growing importance of fish products and plant foods can lead to a significant increase in the production of combined fish and plant products, and, consequently, to an expansion of the range of food products with increased balance [2].

In the last decade, the number of people using ready-made meals and semi-finished products in their diet has increased. In addition, a significant change in the traditional tastes of the population has resulted from increasing awareness of the impact of various products on human health and life expectancy [3, 4].

The development of fish culinary production is able to solve the problem of complex processing of raw materials with reduced market value, traditionally not used by the population for food, as well as secondary products of fish processing and the production of highly nutritious, biologically complete food products from them.

Fish semi-finished products and various culinary products do not require a labor-intensive process of fish processing and after simple processing can be quickly prepared for consumption [5–7].

This type of production is characterized by a large range of goods, which continues to expand constantly. However, the volume of culinary products is limited, since these are mainly perishable products with limited shelf life.

The production of fish pastes in industrial conditions in a wide range allows for a more rational use of fish raw materials compared to the sale of fish as a whole, uncut, chilled or frozen. For example, from large fish it is possible to produce semi-finished products and baked products, from small or mechanically damaged fish – minced and pasty products [8].

The demand for pâté products is steadily growing, with fish pâtés occupying one of the leading places among consumer preferences due to their ease of use, which is especially important in the conditions of the intensive pace of life of modern society. At the same time, the assortment of the Ukrainian market is mostly represented by pâtés made exclusively from fish raw materials without the use of combined ingredients [9]. In this regard, improving technological approaches to the production of pâtés from freshwater fish is a scientifically sound and relevant area of research.

Modern scientific research is actively aimed at optimizing the technology of fish pâtés with the involvement of non-traditional types of raw materials. The main attention is paid to increasing the nutritional and biological value of the product, ensuring its safety and increasing the shelf life.

V. Dorozhko's research [10] was devoted to improving the technology of fish pastes using non-traditional raw materials. The work substantiated the feasibility of combining freshwater fish with plant ingredients, in particular maca root, broccoli and beetroot. The results showed that the addition of these components positively affects the organoleptic characteristics of the product, in particular taste, color and consistency, and also contributes to increasing its biological value. It was established that the use of plant raw materials allows to expand the range of functional food products and improve the overall quality of fish pastes.

In the studies of N. Holembovska and A. Vlasenko [11], the effect of including quail eggs and plant ingredients in the composition of fish pastes on their chemical composition, organoleptic characteristics and physicochemical indicators was analyzed.

V. Sapsay [12] focused on the development of fish paste recipes using plant raw materials in order to increase the nutritional value of the product and improve its organoleptic properties.

In the study of C. Ballo and M. Enriquez, a fish paste was created, manufactured using mechanized equipment for the production of fish paste, and the results of an experimental study of its qualitative and sensory characteristics were presented, which is important for assessing the suitability of such a production technology for the food industry [13].

In the works of N. Holembovska and others [14], the effect of including cranberries and goji berries in the recipe of pâtés on their chemical composition, organoleptic and physicochemical characteristics, and the shelf life of the finished product was determined.

In the work of A. Menchynska et al., new types of fish pastes were developed based on combining fish raw materials with additional ingredients in order to increase their

nutritional and consumer value. The authors found that the use of improved recipes contributes to the improvement of organoleptic indicators and overall acceptability of fish pastes compared to the control sample, which confirms the prospects for the use of non-traditional recipe solutions in the technology of fish products [15].

Other studies show the effect of protein-carbohydrate compositions of plant origin, in particular based on pea and soy proteins, on the quality indicators of fish pastes and found that a rationally selected ratio of plant protein components and fish raw materials contributes to improving the textural characteristics of the product, reducing moisture loss during heat treatment, and enriching the amino acid composition due to essential amino acids [16].

K. Bashir et al. [17] presented natural food additives and preservatives in the technology of fish pastes. The authors systematized modern scientific data on various ingredients used to improve the quality of fish pastes, including the use of various natural additives (for example, seaweed powders, shrimp powder and other components) and ways of their influence on the technological and organoleptic properties of the final products.

In the study of O. Selezneva [18] analyzed the feasibility of using wild animal meat in the technology of meat pâté production, which ensures an increase in their nutritional value and contributes to a decrease in the mass fraction of fat in the finished product.

According to the results of research by I. Bal [19], it was found that the use of pink salmon milt in the technology of fish pastes contributes to the preservation of high organoleptic quality indicators during storage, reduces peroxide value and microbiological contamination, and ensures product safety for up to 120 hours at a temperature of 0–4°C.

Thus, current trends in the development of the food industry indicate the feasibility of improving the production technologies of fish culinary products, in particular pâtés, as a promising direction of rational use of fish raw materials. Their production allows not only to expand the range of products of increased biological value, but also to ensure complex processing of raw materials, including unprofitable fish species and by-products of its processing.

At the same time, the issue of increasing the nutritional and functional value of such products by optimizing the recipe composition, reducing the salt content, using plant components and natural ingredients that meet modern requirements for healthy nutrition remains relevant.

Thus, scientific substantiation of technological solutions for the creation of competitive fish pâtés and other pasty products with specified indicators of quality, safety and balance is an important task of modern food science and practice.

6.2 Scientific substantiation of the functional properties of goji berries (*Lycium barbarum* L.)

Wild berries are widely used in various industries: they are used in the production of finished medicines, as well as in food, canning, confectionery, non-alcoholic and alcoholic, meat and dairy, bakery, perfume and cosmetic products for medical, preventive and health purposes.

Wild plants used as food raw materials are valuable sources of vitamins, minerals and other biologically active components. The creation of new food products using wild berries allows enriching the diet of the population of Ukraine with useful micronutrients. The development of products for medical and preventive nutrition is of particular importance. Adding wild raw materials to food products helps to solve many problems of dietary and health-improving direction. One of the promising ingredients for such purposes can be goji berries.

Goji berries (common dogwood, or Tibetan barberry) belong to the genus Lycium and are part of the nightshade family (*Solanaceae*). It is a perennial evergreen shrub with flexible, drooping branches that can reach about three meters in height. The shoots have thorns, and the leaves are elliptical and short-petiolate. The flowers are purple, bell-shaped, located in the axils of the leaves. The fruits are juicy, bright red, with a characteristic bittersweet or slightly sour taste, elliptical in shape, about 1–2 cm in diameter, and outwardly resemble a small ripe tomato. Each berry contains from 10 to 60 small yellow seeds with a curved embryo. The flowering period falls on September-October, and the fruits ripen in November [20].

Goji berries are found in Japan, Korea, and Eastern China, where they grow along roads, on dry slopes of foothills and mountainous areas. This plant is cultivated in China, Japan, the island of Java, Hawaii, the countries of Southeast Europe, and Asia in general, with the main industrial plantations concentrated in China.

The plant is also widespread in the Mediterranean region, as well as in Southwest and Central Asia. In North America and Australia, goji is often grown as an ornamental or for hedge formation.

The species *L. Chinense* is most characteristic of East Asia and is actively cultivated in South China, Korea and Japan. Industrial cultivation of *L. barbarum* is concentrated mainly in the Chinese region of Ningxia-Hui and in the Xinjiang Uygur Autonomous Region in the west of the country [21].

The genus *Lycium* L. from the nightshade family (*Solanaceae*) has more than 88 species, which are mainly found in non-tropical regions of the world, with the greatest diversity observed in South America. Chinese blackthorn is widespread in Korea, Japan and East China. In natural conditions, it grows in rocky gorges, along

roads, on arid foothills and mountain slopes. This plant is cultivated in Japan and China, as well as on the island of Java, the Hawaiian Islands, in the countries of Central Asia and in Europe.

The high content of biologically active substances, vitamins and mineral components determines the expediency of using berries in the creation of products for medical and preventive and health purposes, especially in conditions of micronutrient deficiency in the diet of the population of Ukraine.

Goji berries, in particular representatives of the genus *Lycium*, are characterized by a valuable chemical composition, a wide distribution area and the possibility of cultivation, which makes them a promising ingredient for the development of new functional food products. At the same time, the insufficient level of scientifically substantiated approaches to the use of goji berries in domestic food production, as well as limited data on the technological aspects of their application, necessitate further research in this direction. This confirms the relevance of the chosen topic and determines the expediency of conducting comprehensive scientific work aimed at substantiating the use of goji berries in medical and preventive nutrition products.

Given the growing body of research supporting the health benefits of natural products [22], global fruit production has increased significantly over the past twenty years [23]. Similar trends are observed for goji berries, with their cultivation areas expanding rapidly in recent years. This is particularly evident in European countries (Italy, Romania, Bulgaria, Portugal, Greece, Serbia), as well as in North America and Australia. Romania currently leads the EU in terms of *L. barbarum* plantation area [24, 25].

According to P. Bora et al. [26], goji berries contain significant amounts of carbohydrates (46 g/100 g of fresh fruit) and dietary fiber (16 g/100 g of fresh weight). In a study by T. Ilić et al. [27], the chemical composition of *L. barbarum* was analyzed and the following indicators were determined: moisture content – 75.32 g/100 g of fresh weight, carbohydrates – 16.93 g/100 g of dry weight, dietary fiber – 3.63 g/100 g of fresh weight, protein – 1.98 g/100 g of dry weight, fat – 1.15 g/100 g of dry weight, and ash – 0.84 g/100 g of dry weight.

Similar data were obtained by T. Pires et al. during the analysis of dried goji berries and stems. The researchers found that their chemical composition includes 87 g/100 g of dry matter carbohydrates, 5.3 g/100 g of protein, 4.1 g/100 g of fat and 3.21 g/100 g of ash. In addition, a significant amount of soluble sugars (27.9 g/100 g of dry matter) was found in the samples, among which fructose (12.7 g/100 g), glucose (14.4 g/100 g) and sucrose (0.8 g/100 g) were identified [28].

In a study by T. Pires et al. [28] on the organic acids of goji berries, the presence of citric (1.29 g/100 g dry weight), succinic (0.77 g/100 g) and oxalic (0.010 g/100 g) acids was found. In addition, the authors identified tocopherols, among which

α-tocopherol (0.23 mg/100 g) and δ-tocopherol (0.09 mg/100 g) were determined. The samples were also analyzed for a fatty acid profile, the total content of which was 4.1 g/100 g dry weight. The researchers found the presence of sixteen types of fatty acids, among which polyunsaturated ones dominated – linoleic (53.4%), oleic (16.5%) and palmitic (12.77%).

According to the results of the study by T. Ilić et al. [27], among the fatty acids in goji berries, linoleic (52.1%), oleic (23.6%) and palmitic (17.6%) dominated, which together account for about 95% of their total content. Similar trends were also noted by P. Skenderidis et al. [29], who recorded the concentrations of linoleic, oleic and palmitic acids in the ranges of 37.89–43.96%, 16.71–20.07% and 15.08–21.79%, respectively.

Regarding the mineral composition, numerous scientific works indicate that the main minerals of goji berries include potassium, sodium and calcium. Thus, according to P. Bora et al. [26], 100 g of berries contain 434 mg of potassium, 60 mg of calcium, 5.4 mg of iron and 1.5 mg of zinc. In the work of E. Llorent-Martínez et al. [30], on the contrary, significantly higher values are given: potassium – 1460 mg/100 g, sodium – 550 mg/100 g and calcium – 50 mg/100 g. In turn, T. Ilić et al. [27], estimated the content of macronutrients in the dry matter of berries: potassium – 445.12 mg/100 g, phosphorus – 231.52 mg/100 g, sodium – 74.57 mg/100 g and calcium – 29.02 mg/100 g. Ascorbic acid (48.94 mg/100 g of fresh fruit) and tocopherols (0.33 mg/100 g of dry weight) were found in goji berries [27]. Vitamin E (α-tocopherol) is a key lipid-soluble antioxidant in cells, capable of inhibiting membrane lipid peroxidation [31, 32], while vitamin C (ascorbic acid) provides important antioxidant activity in goji berries [25].

In the work of M. Polat et al. [33], devoted to the assessment of the quality of goji berries (*Lycium barbarum* L.) depending on the fruiting period and seasonal conditions, it was shown that the physicochemical characteristics of the fruits change during the season. In particular, from the first to the last harvest, a decrease in the length, width and weight of the fruits by 21%, 18% and 33%, respectively, was observed. At the same time, the total anthocyanin content increased by 264%, phenolic compounds – by 48%, and antioxidant activity – by 105%.

The researchers also measured titratable acidity and soluble solids content, and found a positive relationship between the concentration of phenolic compounds and soluble solids and titratable acidity. The results indicate that late harvests provide higher phytochemical value, while early harvests provide better pomological characteristics. In a study by Y. Zhou et al. [34], which focused on the composition, characteristics and antifungal properties of the cutin layer of goji berries (*Lycium barbarum* L.) at different stages of development, it was found that 26 chemical

components were identified in the cutin extracts, among which fatty acids, alkanes, aromatic acids and small molecular acids dominated. These compounds play a key role in the formation of the structure of the cutin layer and may be associated with antifungal activity.

In a study by D. Ağagündüz et al. [35] on the physicochemical and antioxidant profile of dried goji berries (*Lycium barbarum*), it was found that the berries contain high levels of bioactive compounds. Thus, the total phenolic content was 207.2 ± 1.51 mg/100 g dry weight, and the antioxidant activity was 32.6 ± 1.82 μmol/g. The content of L-ascorbic acid (vitamin C) was 31.0 ± 1.62 mg/100 g. In a review by I. Szot et al. [36] on the beneficial and functional properties of goji berries (*Lycium barbarum* and *Lycium chinense*), it was noted that these fruits are rich in various bioactive compounds that provide their antioxidant and functional activities. These include phenolic acids, flavonoids, proanthocyanidins, coumarins, tannins, carotenoids, and anthocyanins, making berries an important source of antioxidants and functional phytonutrients.

In addition to polyphenols, berries contain macro- and micronutrients, including proteins, fats, carbohydrates, and minerals (particularly potassium, magnesium, iron, and zinc), as well as vitamin C. These components play an important role in maintaining health and increasing the nutritional value of berries as a functional product.

Researchers have identified polysaccharides in the amount of 5–8% of the dry weight, which are the main functional components of berries, as well as total phenolic compounds that provide antioxidant activity of fruits.

The fatty acid profile showed the presence of oleic (21.7%), palmitic (8.2%), stearic (2.9%) and myristic (0.1%) acids, among which mono- and polyunsaturated fatty acids dominate [37].

In the study of I. Taneva and Z. Zlatev [38], the mineral composition of berries was analyzed and it was found that 100 g of dried fruits contain: calcium – 49.0 mg, phosphorus – 370.0 mg, sodium – 1.32 mg, potassium – 193.0 mg, magnesium – 120.0 mg, iron – 0.04 mg, copper – 0.01 mg and manganese – 0.008 mg of dry weight.

In the study of M. Spano et al. [39] analyzed the metabolic profile of fresh goji berries (*Lycium barbarum* L.) from two cultivars, Big Lifeberry and Sweet Lifeberry, grown in Central Italy, using an integrated analytical approach combining nuclear magnetic resonance (NMR) and electrospray FT ICR mass spectrometry for the precise detection and quantification of molecules in the fruits. The study showed that the berries contain a wide range of metabolites, including sugars (glucose, fructose, sucrose), amino acids (including glycine, betaine, proline), organic acids, fatty acids, polyphenols, and terpenes, indicating a rich chemical composition and potentially high bioactivity of the fruits. The chemical composition of goji berries includes

betaine, rutin, ascorbic acid, and daucosterol. An essential oil rich in cinnamic acid and phenolic compounds was found in the bark of the plant. In addition, the bark contains leucine, choline, about 2.2% fatty oil, protein substances, daucosterol, and alkaloid compounds characteristic of the Solanaceae family, in particular physalin [40].

Both the fruits and Cortex Lycii have numerous pharmacological properties, including antiglaucoma, immunomodulatory, antitumor, antioxidant, anti-aging, neuroprotective activity, and the ability to lower blood sugar levels. For many centuries, goji berries have been used in Asia as a medicinal plant raw material due to their high nutritional value, health-promoting properties, and a wide range of biological activities [26, 41]. Several scientific studies confirm their usefulness, including antioxidant activity [24, 42, 43], antitumor effect [24, 44, 45], antimicrobial properties [44, 46], the ability to lower blood glucose [25] and lipid levels [25], as well as antimutagenic [45], prebiotic [25, 26, 46], immunomodulatory [47], antifatigue [44], antiaging and neuroprotective effects [43].

Approximately 75–85% of fresh goji berries after harvest undergo a dehydration process, most often carried out using traditional heat drying, freeze-drying or vacuum-pulsation methods, before they are sold [48–50]. In addition to dried fruits, other goji products are widely available on the market, including juices, wines and various primary processed products.

Among the berry beverages, pulp juice, clear juice, dry instant drinks, as well as dairy and sour-milk products made by fermentation with lactic acid bacteria are offered [51–54]. Wine products include blended wines obtained by infusing berries and other bioactive or medicinal components in strong alcoholic beverages, as well as fermented wines, where goji berries are fermented together with dates (*Ziziphus jujuba Mill.*), honey and other nutritional additives [55].

Wild berry crops are considered as a promising source of raw materials for the production of health and medical and prophylactic food products, which is explained by the high content of biologically active substances and a wide range of their use in various industries. Of particular interest among such plants are goji berries (*Lycium spp.*), which have been used in traditional Asian medicine for centuries and are increasingly being included in modern food technologies.

Analysis of scientific data shows that goji berries are characterized by high nutritional value and complex biochemical composition. They contain carbohydrates, dietary fiber, proteins and lipids, organic acids, antioxidant vitamins (ascorbic acid and tocopherols), as well as macro- and microelements. The predominance of polyunsaturated fatty acids, in particular linoleic, enhances their biological potential. Taken together, these components provide antioxidant, immunomodulatory, metabolic-corrective, neuroprotective and geroprotective properties of goji berries.

Therefore, goji berries can be considered as a promising functional ingredient for the creation of new food products with increased biological value. Further scientific research aimed at improving processing technologies in order to preserve bioactive components and develop innovative products from wild plant raw materials remains relevant and has significant practical importance for the development of the food industry of Ukraine.

6.3 Characteristics of the nutritional and biological value of fish and vegetable raw materials

Fish fillets must meet certain organoleptic and physicochemical characteristics, which ensures the quality of the final product. Analysis of organoleptic indicators of goji berries allows to assess their taste, color and consistency properties, and the chemical composition allows to determine the content of macro- and microelements, proteins, pectin substances and fiber, which justifies their functional value in nutrition.

The obtained data on the organoleptic and chemical characteristics of fish and berry components allow to plan the technological process of producing fish pastes with high nutritional and biological value, which makes them promising as a functional food product.

According to organoleptic indicators, frozen fillets must meet the requirements and standards specified in **Table 6.1**.

The primary raw materials for cooking fish pastes are minced crucian and crushed goji berries.

The dimensional composition of the fish is given in the **Table 6.2**.

The length of the carcass is 25.2 cm, the height of the fish body is 6 cm, and the width of the fish body is 4 cm (average size of the fish). The mass composition of crucian is presented in **Table 6.3**.

The output of fish meat is 25.9 g, waste – 66.1 g, losses – 7.9 g. The chemical composition of fish raw materials was determined during the study, as shown in **Table 6.4**.

The results of studies of organoleptic indicators of cranberries and goji berries are presented in **Table 6.5**.

The chemical composition of goji berries is presented in the **Table 6.6**.

Analyzing the presented data, it is possible to conclude that goji berries are characterized by low moisture content (12.3%), which contributes to their longer shelf life. The content of pectin substances (2.5%) and fiber (3.6%) indicates the ability of the berries to support digestion and normalize intestinal function.

Table 6.1 Organoleptic characteristics of frozen fish fillets

Indicator name	Characteristic and standard
Appearance: blocks individually frozen fillets	Clean, dense, with a flat surface without significant differences in block height. Clean, even, whole, without significant deformation. May exhibit: slight loosening of the muscle tissue along the edge of the fillet block; presence of scale residues on the surface of the fillet with skin without scales; skin damage in horse mackerel and sturgeon fillets at the sites where scutes have been removed
Placement procedure	The fillets are placed into molds in uniform layers: in the bottom layer with the skin or subcutaneous side facing downward, and in the top layer with the skin or subcutaneous side facing upward
Flesh consistency: after defrosting	Firm, typical of this type of fish
After boiling	Tender, juicy, brittle, typical of this type of fish. It may be slightly dry, fibrous, but not hard, rubbery, jelly-like
Flesh color	Typical of this type of fish
Odor (after defrosting)	Typical of fresh fish, without any foreign odor
Taste and smell after cooking	Typical for this type of fish, without any foreign taste or smell

Table 6.2 Dimensional composition of crucian

L_a, cm	L_i, cm	L_h, cm	L_t, cm	L_m, cm	h, cm	b, cm
23	18.5	4.5	5	14	7	2

Note: initial weight of gutted carcass – 356 g

Table 6.3 Mass composition of crucian

Weight, kg	Content to the total weight of fish, %				
	fillet	skin	bones	fins	scales
0.177	25.9 ± 0.5	3.9 ± 0.3	12.7 ± 0.9	4.6 ± 0.2	7.9 ± 0.2

Note: initial weight of gutted carcass – 177 g, results are in %, ($n = 5$, $p \leq 0.05$)

Table 6.4 Chemical composition of hake

Indicator	Content
Protein content	16.5 ± 0.5
Fat content	3.9 ± 0.1
Moisture content	78.5 ± 1.3
Mineral content	1.1 ± 0.15

Note: results are in %, ($n = 5$, $p \leq 0.05$)

Table 6.5 Organoleptic characteristics of goji berries

Indicators	Goji berries
Appearance and consistency	The berry is dark red, spherical or ellipsoidal in shape, up to 12 mm in diameter, without visible inclusions or impurities
Taste and smell	Pleasant juicy, sour taste with a slightly bitter aftertaste
Color	Dark red, uniform throughout the mass

Table 6.6 Chemical composition of goji berries

Indicator name	Goji berries
Mass fraction of moisture, %	12.3 ± 0.2
Pectic substances, %	2.5 ± 0.1
Fiber, %	3.6 ± 0.2
Essential macronutrients, mg/kg	
K	2265 ± 23.22
Ca	888.1 ± 8.88
Mg	1357 ± 13.6
Zn	10.33 ± 0.8
Essential micronutrients, mg/kg	
Fe	91.58 ± 0.92
Mn	9.82 ± 0.2

Among the macronutrients, the most abundant are potassium (2265 mg/kg), magnesium (1357 mg/kg) and calcium (888.1 mg/kg), which makes the berries useful for the cardiovascular system and bones. The content of zinc (10.33 mg/kg) also supports immunity.

Among the micronutrients, the most significant is iron (91.58 mg/kg), which helps prevent anemia, and manganese (9.82 mg/kg) supports antioxidant processes in the body.

Thus, goji berries are a valuable source of both macro- and micronutrients, as well as dietary fiber, which justifies their use as a functional food product.

6.4 Technological aspects of the production of fish pastes with the addition of goji berries

The following materials were used to make minced meat and ready-made pastes: crucian carp grown in the Kyiv region, Goji berries produced by LLC "NVO FitoBio-Technology", Organic Herbs.

The crucian carp were stored in a refrigerator at a temperature of +4°C for one day until the experiment.

At the initial stage, all the necessary ingredients were prepared, including crucian carp, Goji berries, carrots, onions, oil, salt and ground black pepper.

The prepared fish carcasses are cleaned, disassembled, while removing inedible parts of the fish: caudal, dorsal and anal fins, gill covers, eyes, intestines and gall bladder. All edible parts of the fish are used to obtain the product. Then the disassembled fish is washed in running water, small fish are whole, and large fish are cut into pieces.

The disassembled fish is submitted for blanching, the purpose of which is to destroy enzymes, increase the permeability of cell protoplasm, which is necessary to improve the taste, reduce the amount of microflora, partially remove air from the raw materials, and oxygen. After blanching, cooling was carried out to a temperature in the thickness of the product of 20–30°C.

The chopped vegetables are submitted to a perforated vibrating roller, where the product is dehydrated for 3–4 minutes, then the vegetables are sent to a bath with oil heated to 95–100°C, where the vegetables are saturated with oil, then the vegetables are submitted to a vibrating tray, on which the vegetables are fried at an oil temperature in the range of 95–100°C.

Then the blanched crucian carp, onions and carrots are chopped into a shredder with a grate diameter of ∅ = 4 mm, which ensures uniform grinding of the raw materials. To conduct the experiment, 2, 4, 6% of Goji berries were added to the recipes of the test samples. Dried Goji berries before use in the production of pâtés are subject to preliminary preparation in order to restore their structure, reduce stiffness, ensure microbiological safety and uniform distribution in the product.

At the first stage, the berries are sorted to remove foreign impurities and damaged berries. Then the berries are washed in running drinking water to remove surface contaminants.

The next stage is hydration (soaking) of dried berries when they are poured with warm water at a temperature of 40–60°C in a ratio of 1:5 and kept for 30 minutes until the pulp is fully elastic.

After hydration, a short-term heat treatment is carried out, blanching for 1–2 minutes, in order to reduce the microbiological load and stabilize the color of the raw materials. Then the berries were ground to the required degree of dispersion using a laboratory mill grinder SM-3C for the desired consistency of the pate.

Prepared Goji berries are introduced into the pate mass at the cutting stage. The mass fraction of Goji berries is 2, 4, 6% of the total mass of the pate, taking into account the recipe features.

All ingredients were weighed and homogenized until a uniform consistency was achieved, packed into containers and subjected to heat treatment in boiling water (100°C) for 60 minutes.

6.5 Fish paste recipes

Modern consumers are increasingly paying attention to food products not only in terms of taste, but also taking into account their functional and biological value. The growing demand for healthy eating stimulates the development of the market for fish culinary products, in particular, pâtés and paste-like products, which combine high nutritional value with ease of consumption.

Solving these problems is possible by including plant components in the recipe, which not only improve the organoleptic properties of the product, but also increase its functional value. Among the promising ingredients are carrots, onions and goji berries, which are characterized by a high content of biologically active compounds, minerals and dietary fiber. Goji berries in particular are distinguished by their antioxidant activity, richness in trace elements (potassium, magnesium, calcium, iron, zinc) and the ability to increase the shelf life of products.

The aim of the study is to assess the impact of introducing plant components, in particular goji berries, on the physicochemical, organoleptic and functional properties of fish pastes, as well as to develop a product formulation with an optimal ratio of fish and plant raw materials. The implementation of such approaches allows creating a competitive food product that meets modern requirements for healthy nutrition and consumer needs.

Taking into account the standards of need recommended by FAO/WHO, formulations of new pastes were developed. Samples of pastes were selected taking into account the content of the main components in them: experiment 1 – with the addition of 2% berries; experiment 2 – with the addition of 4% berries, experiment 3 – with the addition of 6% berries, the control sample – without additives, based only on crucian carp meat.

The developed formulation of the control sample is presented in **Table 6.7**.

Combining freshwater fish raw materials with vegetable raw materials allows to optimize the taste properties of the finished product, biological value and extend the shelf life.

The recipes for new crucian carp-based pastes are given in **Table 6.8**.

Analysis of the formulation composition showed that the introduction of plant raw materials (carrots, onions and goji berries) allows to partially reduce the share

of fish raw materials without significantly disrupting the structure of the product, while increasing its functional value. Goji berries are a source of biologically active substances, antioxidants, vitamins and minerals, which contributes to increasing the nutritional and biological value of pâtés.

Table 6.7 Recipe of the control sample of pate

Component names	Formulation composition, kg per 100 kg of product
	Natural pate (Control)
Stuffed crucian carp	88
Salt	1.5
Ground black pepper	0.5
Sunflower oil	10

Table 6.8 Recipes for pâtés with vegetable additives

Component names	Formulation composition, kg per 100 kg of product		
	Sample 1	Sample 2	Sample 3
Stuffed crucian carp	69	67	65
Salt	1.5	1.5	1.5
Ground black pepper	0.5	0.5	0.5
Sunflower oil	10	10	10
Carrot	8	8	8
Onion	9	9	9
Goji berries	2	4	6

Combining freshwater fish raw materials with plant components provides: improvement of organoleptic indicators (taste, aroma, color); enrichment of the product with dietary fiber and antioxidant compounds; potential increase in resistance to oxidative processes and expansion of the range of functional fish products.

Thus, the developed formulations are promising in terms of increasing nutritional value, forming new taste characteristics and creating a competitive functional product based on freshwater fish. It is advisable to determine the optimal percentage of berries added based on the results of further organoleptic, physicochemical, and microbiological studies.

6.6 Chemical composition of fish pastes

In modern food production, special attention is paid not only to the taste properties of products, but also to their nutritional and functional value. One of the directions for increasing the nutritional value of finished products is the introduction of plant components, in particular berries and vegetables, which enrich products with biologically active substances and minerals.

The study of the chemical composition of fish pastes with the addition of goji berries was carried out in order to assess the influence of plant ingredients on the main nutritional indicators of the product: protein, fat, moisture and mineral content. The analysis allows to determine how changing the ratio of minced fish and additives affects the nutritional value and technological properties of products, and also allows to choose optimal recipe solutions for the production of functional food products.

The results are presented in **Tables 6.9**, **6.10** and demonstrate changes in the main components and mineral composition of fish pastes under the influence of the introduction of goji berries, which allows to draw conclusions about their effectiveness as a functional ingredient.

Table 6.9 General chemical composition of semi-finished products, % ($n = 5$, $p \leq 0.05$)

Name of indicators	Fish pâté			
	Control	Sample 1	Sample 2	Sample 3
Moisture content	70.38 ± 1.5	68.86 ± 0.24	68.72 ± 0.3	68.69 ± 0.33
Protein content	15.84 ± 0.65	14.83 ± 0.88	14.71 ± 0.71	14.59 ± 0.43
Fat content	12.52 ± 0.05	13.39 ± 0.07	13.42 ± 0.05	13.47 ± 0.05
Mineral content	1.26 ± 0.04	2.92 ± 0.04	3.15 ± 0.05	3.25 ± 0.06

The analysis of the chemical composition of the control sample and three samples of fish pastes with the addition of vegetables and goji berries showed that the introduction of plant components affects the nutritional properties of the product. Reducing the proportion of minced fish led to a slight decrease in protein content (from 15.84% in the control to 14.59% in the sample with 6% goji) and a simultaneous increase in fat content (from 12.52% to 13.47%). Humidity remained practically stable, indicating the preservation of consistency and optimal technological properties of the pastes. At the same time, increasing the proportion of goji berries contributed to an increase in the content of minerals (up to 3.25%), which increases the functional value of the product. The results obtained allow to recommend the sample with

6% goji berries as optimal in terms of the combination of nutritional value, mineral composition and technological characteristics.

6.7 Research on organoleptic indicators of fish pastes

In order to determine the taste properties of fish pastes, an organoleptic assessment of the quality of the test samples was carried out during all stages of production and storage. The assessment was carried out using a self-developed 5-point scale.

To assess the sensory properties of products, the flavor profiling method is used, which belongs to the basic descriptive methods of sensory analysis [56]. This approach is widely recommended for the development of new and improvement of existing food products [57]. The use of the flavor profiling method allows for a detailed description of the set of descriptors that determine the overall sensory impression of the product. The advantage of sensory analysis compared to instrumental methods is the possibility of simultaneous human assessment of a wide range of organoleptic indicators and their integrated interpretation.

The method described in DSTU ISO 6564:2005 "Sensory research. Methodology. Methods for creating a flavor spectrum" [58, 59] was used to create the flavor profiles. The sensory evaluation was carried out by 20 permanent members, including teachers, staff and postgraduate students. The evaluators tasted and rated the pâtés for appearance, aroma, color, taste, viscosity and overall acceptability. The rating scales were provided in the evaluation sheet to all evaluators. Regarding taste and mouthfeel, the evaluation was carried out by analyzing the harmony of taste, aftertaste, tenderness and juiciness of the product. Regarding aroma, appearance and viscosity, the fish pâté was placed in a saucer, smelled and observed visually. It was allowed to smell and taste again. The procedure was repeated three times with an interval of 5 minutes. Participants of the experiment rinse their mouths in preparation for the next test.

For the sensory assessment of fish pastes, the survey participants were offered a scale of ten descriptors, ordered in descending order of their significance. Based on the analysis of the descriptors, their weight significance in the formation of an integral assessment of the quality of the samples was determined, taking into account the consumer importance of each indicator. The tasting assessment was carried out on a scale of desirability and intensity of the taste and aromatic characteristics of the product, where 0 points corresponded to the absence of a sign, 1 point – barely noticeable intensity, 2 points – weak manifestation, 3 points – medium level, 4 points – strong and 5 points – very strong manifestation of the indicator [60].

Laboratory results data were presented as the mean standard deviation and statically analyzed using one-way analysis of variance to determine significant differences between groups. All statistical analyses were performed using statistical analysis programs in Excel.

Based on the results of consumer preference studies that were conducted, a set of 10 descriptors was defined for flavor characterization (**Table 6.10**).

Table 6.10 Sensory evaluation of fish pastes using the flavor profile method ($n = 5$, $p \leq 0.05$)

Descriptors	Intensity of characteristics, score				
	Fish pâté				
	Standard	Control	Sample 1	Sample 2	Sample 3
Aroma and taste characteristics					
harmonious	5.0	4.0 ± 0.10	5.0 ± 0.10	5,0 ± 0.20	5.0 ± 0.20
typical	4.5	3.0 ± 0.01	4.0 ± 0.01	4.0 ± 0.02	4.0 ± 0.02
fishy	4.5	3.5 ± 0.10	4.5 ± 0.10	4.5 ± 0.10	4.8 ± 0.30
slightly pronounced	3.5	1.0 ± 0.20	3.0 ± 0.10	4.1 ± 0.20	3.5 ± 0.10
sweet	3.0	1.0 ± 0.01	2.5 ± 0.20	2.8 ± 0.30	3.0 ± 0.03
salty	3.0	3.0 ± 0.01	2.5 ± 0.01	2.5 ± 0.10	2.5 ± 0.02
Consistency characteristics					
tender	3.0	3.0 ± 0.10	2.0 ± 0.20	2.5 ± 0.20	2.5 ± 0.10
juicy	3.5	3.0 ± 0.10	3.5 ± 0.20	3.5 ± 0.10	3.0 ± 0.10
spreadable	1.0	2.0 ± 0.02	2.5 ± 0.02	3.0 ± 0.02	2.9 ± 0.02
Overall impression	5.0	4.4 ± 0.10	5.0 ± 0.20	5.0 ± 0.10	4.0 ± 0.10
Total points	36.0	27.9	34.5	36.9	35.2

Analyzing this table, it is possible to observe that the control sample is characterized by reduced indicators of harmony of aroma and taste (4.0 points), a weakly expressed fishy taste (3.5 points) and insufficient intensity of sweet and salty notes (1.0–3.0 points each). The consistency is juicy and plastic (3.0 points each), but the density slightly exceeds the reference value (2.0 points versus 1.0). The overall impression of the control sample is 4.4 points, and the total score is only 27.9 points, indicating lower consumer properties than the reference.

In experimental sample 1, there is a significant improvement in sensory characteristics. The indicators of harmony and fishy taste have reached the reference level (5.0 and 4.5 points, respectively). Also, moderate flavor intensity (3.0 points) was noted, with sweet and salty notes balanced (2.5 points), and optimal plasticity indicators (3.5 points). The overall impression was 5.0 points, and the total score was 34.5 points, which significantly exceeded the control.

Experimental sample 2 is characterized by the most harmonious combination of aroma, taste, and consistency. High scores were given to harmony (5.0 points), moderately pronounced taste (4.1 points) and optimal density (3.0 points). Juiciness and plasticity indicators corresponded to the reference values or close to them. The total number of points for this sample is the highest – 36.9 points, which even exceeds the reference (36.0 points), and the overall impression is 5.0 points.

Experimental sample 3 is also characterized by high sensory indicators, in particular, an intense fishy taste (4.8 points) and harmony (5.0 points). At the same time, a slightly lower overall impression score (4.0 points) and reduced juiciness (2.5 points) caused a slight decrease in the total score to 35.2 points compared to experimental sample 2.

The results obtained indicate that the use of improved recipes and technological parameters in the experimental samples allowed to significantly improve the organoleptic properties of fish pastes. The most optimal in terms of the set of sensory indicators is experimental sample 3, which is characterized by high harmony of taste and aroma, balanced consistency and the highest sum of points. This confirms the feasibility of the selected technological solutions for the production of fish pastes of increased biological value.

The control sample of fish pastes was distinguished by a light gray color, a sweet-salty taste, as well as a plastic and uniformly dense consistency. Such indicators indicate the need to improve the recipe in order to achieve the desired sensory characteristics (**Fig. 6.1**).

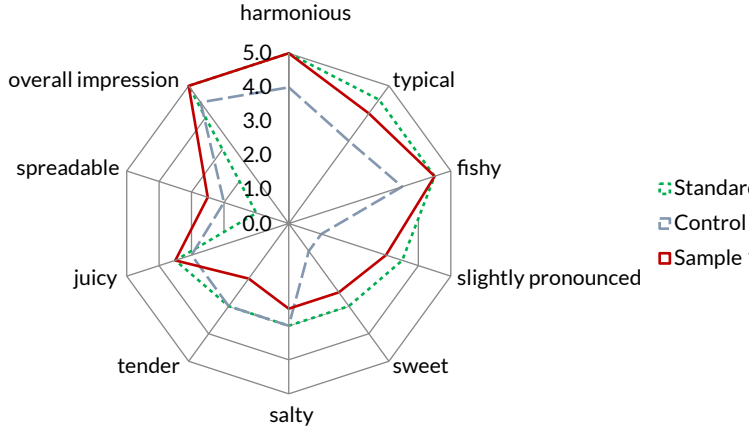

Fig. 6.1 Flavor profile of fish pâté with the addition of Goji berries (sample 1)

The figure shows the results of the organoleptic evaluation of the samples, presented in the form of a petal diagram. This method of visualization allows to clearly trace the differences between the reference, control and experimental samples according to the main sensory indicators. The evaluation was carried out taking into account the harmony of taste, typicality of aroma, pronounced fishy aftertaste, intensity of taste, sweetness, saltiness, juiciness, plasticity, density of consistency and overall impression.

As can be seen from the diagram, the reference sample has the most balanced profile and received the highest scores for the indicators "harmonious", "characteristic" and "overall impression". This indicates its good sensory quality and compliance with the expected characteristics of the product. The control sample is close to the reference in most indicators, but is somewhat inferior in individual taste and textural properties.

The experimental sample is characterized by certain differences in the profile. In terms of individual taste indicators, it approaches the reference, but in terms of consistency characteristics, in particular density and plasticity, there is a decrease in the ratings. At the same time, the overall impression of the sample remains at a sufficient level, which indicates its potential consumer appeal.

When comparing the calculated overall score in points, the experimental sample exceeds the reference with the addition of goji berries in the amount of 4% – with a score of 36.9 (**Fig. 6.2**).

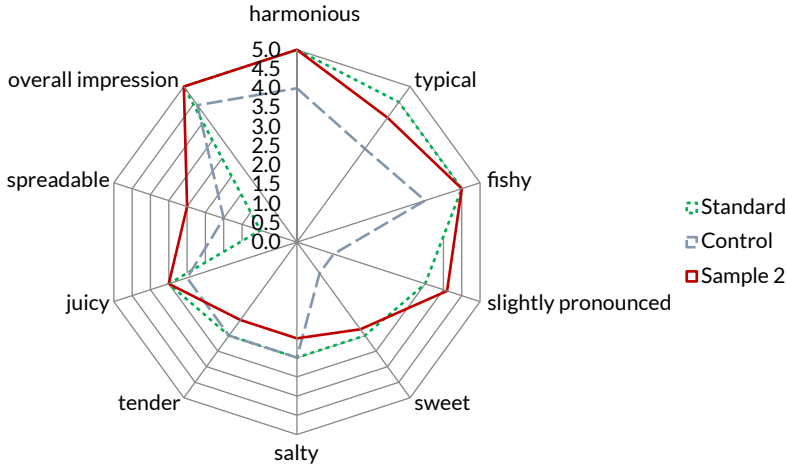

Fig. 6.2 Flavor profile of fish pâté with the addition of goji berries (sample 2)

Experimental sample 2 generally demonstrates positive dynamics compared to the control. In particular, sufficient harmony of taste, a well-pronounced fishy aftertaste and better plasticity indicators were noted. At the same time, its density indicator score is lower than that of the reference sample, which may indicate a softer product structure. Sweetness and saltiness are moderate and do not disrupt the overall taste balance.

Thus, the results obtained indicate that experimental sample 2 exceeds the reference and control indicators in most characteristics. This confirms that the product, according to this recipe, is the most balanced.

The flavor profile of the fish pate of experimental sample 3 is shown in **Fig. 6.3**.

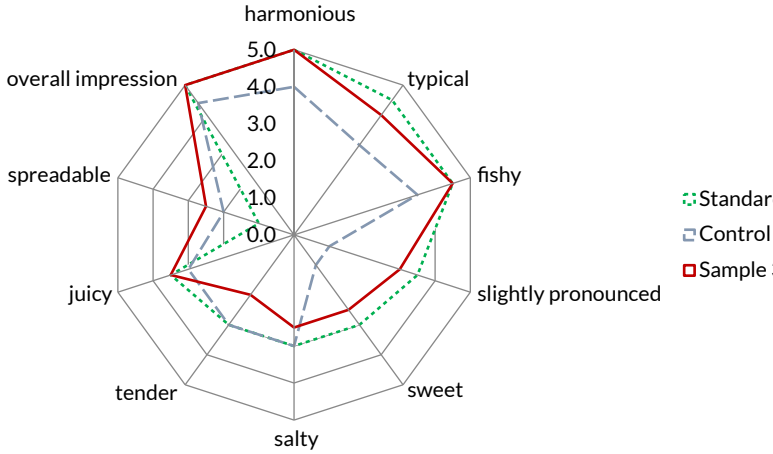

Fig. 6.3 Flavor profile of fish pâté with the addition of goji berries (sample 3)

Experimental sample 3 shows a tendency to improve individual indicators compared to the control, particularly in taste harmony, fishy flavor, and plasticity. At the same time, its values for the "density" parameter remain lower, suggesting a less dense, softer product consistency. The indicators of sweetness and saltiness are within moderate values and do not create a taste imbalance.

Thus, the results obtained indicate that experimental sample 3 approaches the reference in most sensory characteristics and exceeds the control in individual indicators. This confirms the positive impact of the technological changes introduced and the feasibility of further optimizing them to improve the product's structural and mechanical properties.

6.8 Dynamics of organoleptic, physicochemical quality indicators of fish pastes during storage

Organoleptic evaluation is one of the key methods for determining the quality of food products, since sensory indicators directly shape consumer preferences and product competitiveness. For fish pastes, appearance, color, aroma, taste, and consistency are particularly important and can change during storage due to physicochemical and microbiological processes.

The introduction of plant raw materials, in particular goji berries, can not only increase the biological value of the product, but also affect its sensory characteristics and stability during storage. In this regard, it is relevant to study the dynamics of organoleptic indicators of fish pastes with different contents of plant additives during the established storage period.

The aim of this study was to determine the effect of goji berries on the organoleptic properties of fish pastes and establish the optimal storage period based on the results of tasting evaluation.

Studies of organoleptic quality indicators of experimental samples of fish pastes during the storage period are given in **Table 6.11**.

Table 6.11 Organoleptic evaluation of fish pastes based on vegetable raw materials, scores ($n = 7$, $p \leq 0.05$)

Sample name	Shelf life, days	Indicators					Total score
		Appearance	Taste	Scent	Color	Consistence	
Control	1	4.1 ± 0.3	3.8 ± 0.2	4.0 ± 0.3	4.3 ± 0.3	4.1 ± 0.3	20.3
	2	4.2 ± 0.3	4.0 ± 0.3	4.1 ± 0.3	4.3 ± 0.3	4.1 ± 0.3	20.7
	3	4.2 ± 0.3	4.1 ± 0.3	4.1 ± 0.3	4.3 ± 0.3	4.1 ± 0.3	20.8
	4	4.2 ± 0.3	3.3 ± 0.3	4.0 ± 0.3	3.4 ± 0.3	4.2 ± 0.3	19.1
Sample 1	1	4.7 ± 0.4	4.3 ± 0.3	4.4 ± 0.3	4.4 ± 0.3	4.5 ± 0.4	22.3
	2	4.7 ± 0.4	4.5 ± 0.4	4.5 ± 0.4	4.7 ± 0.4	4.7 ± 0.4	23.1
	3	4.7 ± 0.4	4.6 ± 0.4	4.5 ± 0.4	4.7 ± 0.4	4.7 ± 0.4	23.2
	4	4.8 ± 0.4	4.4 ± 0.4	4.4 ± 0.4	4.6 ± 0.4	4.7 ± 0.4	22.9
Sample 2	1	4.7 ± 0.4	4.5 ± 0.4	4.5 ± 0.4	4.7 ± 0.4	4.3 ± 0.4	22.7
	2	4.8 ± 0.4	4.6 ± 0.4	4.6 ± 0.3	4.8 ± 0.4	4.4 ± 0.3	23.2
	3	4.8 ± 0.4	4.6 ± 0.4	4.6 ± 0.3	4.8 ± 0.4	4.5 ± 0.3	23.3
	4	4.8 ± 0.4	4.6 ± 0.3	4.5 ± 0.4	4.8 ± 0.4	4.4 ± 0.3	23.1
Sample 3	1	4.7 ± 0.4	4.5 ± 0.4	4.5 ± 0.4	4.9 ± 0.4	4.3 ± 0.4	22.9
	2	4.3 ± 0.3	4.6 ± 0.4	4.6 ± 0.3	4.7 ± 0.4	4.4 ± 0.3	22.6
	3	4.3 ± 0.3	4.6 ± 0.4	4.6 ± 0.3	4.7 ± 0.4	4.5 ± 0.3	22.7
	4	4.3 ± 0.3	4.6 ± 0.3	4.5 ± 0.4	4.6 ± 0.4	4.4 ± 0.3	22.4

As shown in **Table 6.11**, the best organoleptic indicators are those of fish pastes stored for 3 days. It is during this period that consumer properties form. The product acquires the best aroma, a delicate consistency, and a pleasant taste. After the tasting evaluation, it was found that all fish paste samples during this period had an attractive appearance, a pleasant taste and smell, and a fairly delicate, juicy consistency. According to the organoleptic evaluation, the best results were obtained with fish pastes containing goji berries. The control sample received the lowest score.

After 3 days of storage, the product's taste deteriorated, and an unpleasant odor developed.

During storage, a gradual increase in the acid and peroxide numbers was observed in all test samples, indicating the progression of hydrolytic and oxidative processes in the product's lipid fraction. An increase in the acid number characterizes the accumulation of free fatty acids due to the hydrolysis of triglycerides. In contrast, an increase in the peroxide number reflects the initial stages of lipid oxidation with the formation of peroxides and hydroperoxides.

The physicochemical parameters of the samples were studied during the storage period at a temperature of 0°C to 5°C in comparison with the control samples.

The depth of oxidative and hydrolytic changes in lipid substances of frozen semi-finished products during storage was estimated by the acid and peroxide numbers (**Fig. 6.4**, **6.5**).

The figure shows the change in the acid number (mg KOH) of the experimental fish paste samples during storage. All variants exhibit an exponential trend in the indicator's growth, as confirmed by high values of the coefficient of determination.

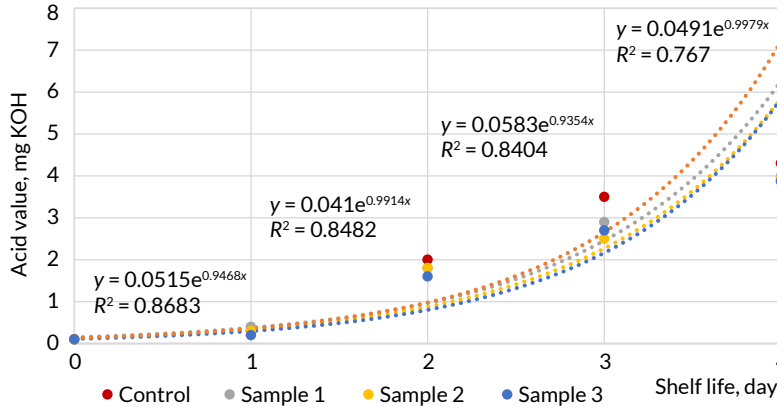

Fig. 6.4 Dynamics of changes in the acid value of fat in fish pastes during storage at 4°C

In the first 0–1 day of storage, the acid number remained at a minimum level and did not differ significantly between the samples. Starting from the second day, a gradual acceleration of its growth was observed, while on the 3–4th day a more intensive increase in the indicator was noted.

Throughout the entire period, the highest values of the acid number were established in the control sample. In contrast, samples with the addition of plant raw materials were characterized by a slower accumulation of free fatty acids. The smallest increase in the indicator was recorded in the variant with a higher proportion of functional additive.

The data obtained indicate that during storage, hydrolytic cleavage of lipids occurs, as evidenced by the exponential growth of the acid number. At the same time, the introduction of goji berries slows down the course of these processes, which is probably due to the presence of natural antioxidants in their composition. Thus, the use of plant raw materials contributes to increasing the stability of the lipid fraction of pates and can ensure the extension of their shelf life. The most pronounced stabilizing effect was observed in the sample with a larger amount of goji berries.

From the data presented in **Fig. 6.5**, it was established that changes in the peroxide value of lipids of semi-finished products during storage have a linear tendency to increase, which indicates the accumulation of primary oxidation products – peroxides. In the control cutlet samples, fat hydrolysis products accumulated more intensively than in the experimental samples.

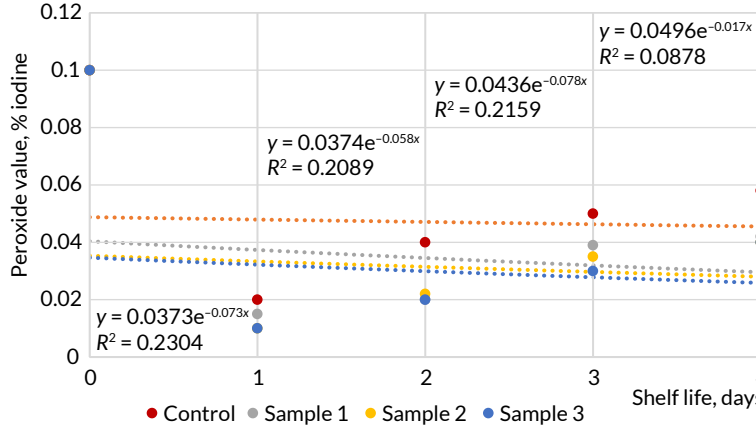

Fig. 6.5 Dynamics of changes in the peroxide value of fat in fish pastes during storage at 4°C

Analysis of the dynamics of the peroxide value in fish pastes with the addition of goji berries over 4 days of storage indicates a gradual increase in the indicator in all experimental samples, a natural consequence of lipid oxidation.

The lowest values of the peroxide value throughout the entire storage period were observed in samples 2 and 3, which indicates a pronounced antioxidant effect of goji berries.

In order to extend the shelf life, the paté samples were placed in plastic containers, which were hermetically sealed to avoid drying and oxidation of the fat. After that, they were stored at a temperature of −12°C and the determination of the chylo and peroxide value was carried out throughout the entire shelf life (**Fig. 6.6**, **6.7**).

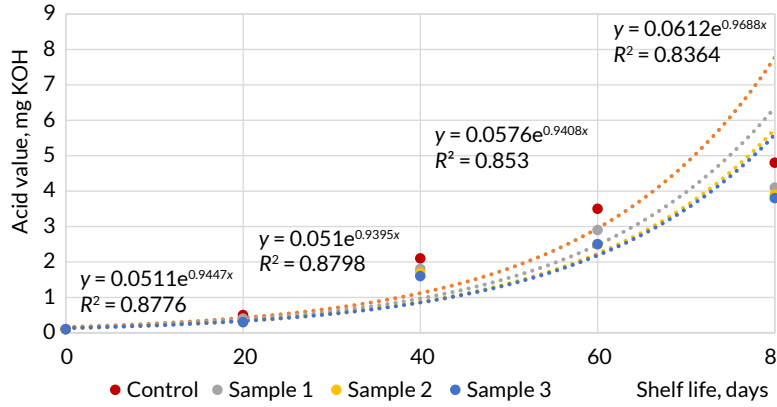

Fig. 6.6 Dynamics of changes in the acid value of fat in fish pastes during storage at −12°C

According to the results of the study, during storage, there is a gradual increase in the acid number in all the studied samples, which indicates the course of the processes of hydrolytic cleavage of lipids and the accumulation of free fatty acids.

Therefore, in the control sample, the accumulation of free fatty acids occurs most rapidly, while in the experimental samples a slower rate of increase in acid number is observed.

During storage, an exponential increase in the peroxide value is observed, which is due to the development of lipid oxidation processes. At the same time, in the experimental samples, the intensity of accumulation of primary oxidation products is lower compared to the control, which indicates an increase in the oxidative stability of the product and an extension of its shelf life.

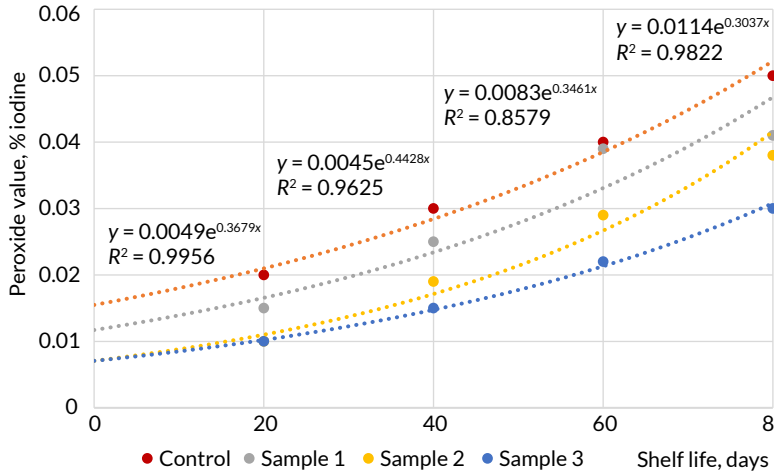

Fig. 6.7 Dynamics of changes in the peroxide value of fat in fish pastes during storage at 12°C

Based on the conducted studies, it was found that the storage temperature significantly affects the intensity of hydrolytic and oxidative processes in fish pastes. During storage at a temperature of 4°C, a faster increase in the acid and peroxide numbers is observed, which indicates a more intense accumulation of free fatty acids and primary lipid oxidation products.

In contrast, storage at a temperature of −12°C provides a much slower increase in the studied indicators. This is explained by the slowdown of biochemical and oxidative processes in the fat fraction of the product at a lower temperature.

The results obtained are consistent with data on the antioxidant properties of *Lycium barbarum*, which fruits contain polyphenols, carotenoids, and ascorbic acid, which are capable of inhibiting lipid peroxidation.

Thus, the addition of goji berries to the formulation of fish pastes increases their oxidative stability and slows the accumulation of primary fat oxidation products during storage, thereby positively affecting the product's quality and potential shelf life.

6.9 Microbiological indicators of fish pastes

To establish the microbiological safety of a new type of minced meat with vegetable raw materials, the total number of mesophilic aerobic and facultative

anaerobic microorganisms in 1 g of the product, the presence of *Escherichia coli* bacteria, and pathogenic organisms were experimentally determined (**Table 6.12**). Pate samples were selected for analysis after 3 days of storage.

Table 6.12 Microbiological quality indicators of fish pastes

Naming indicators	Permissible level	Shelf life, days	Samples of fish pastes			
			Control	Sample 1	Sample 2	Sample 3
MAFAnM, CFU in 1 g	No more 2×10^4	3	1.0×10^5	1.2×10^4	1.3×10^4	1.3×10^4
BECG (coliforms), in 0.1 g	Not allowed	3	No	No	No	No
Golden Staphylococcus, in 0.1 g	Not allowed	3	No	No	No	No
Pathogenic microorganisms, including *Salmonella*, in 25 g	Not allowed	3	No	No	No	No

In the control samples after storage of fish pastes, an increase in KMAFAnM was noted compared to the experimental samples, which indicates the influence of algae on the extension of the shelf life of semi-finished products.

Microbiological indicators of the control and experimental samples throughout the entire storage period meet regulatory requirements, indicating the epidemiological safety of the produced fish pastes.

6.10 Conclusion

Analysis of current trends in the market of fish culinary products indicates a growing need for products with increased nutritional and biological value. Traditional types of products, such as canned, salted, and smoked fish, are limited due to the high content of table salt, carcinogenic substances from smoking, and imperceptible undesirable changes during sterilization. At the same time, the development of fish culinary production enables the comprehensive processing of fish with reduced commercial value, secondary products, and small fish, creating pâtés and pasty products that do not require complex processing and meet modern consumer requirements.

Studies have shown that the introduction of plant raw materials, in particular carrots, onions, and goji berries, into the recipe of fish pâtés allows for an increase

in their nutritional, biological, and functional value, enriching the product with antioxidants, vitamins, macro- and microelements. Goji berries support the digestive system, improve mineral balance, and contribute to antioxidant and immunomodulatory effects, making them a promising ingredient for the creation of functional food products.

The inclusion of plant components, in particular carrots (8%), onions (9%), and goji berries (2–6%), allows to partially reduce the share of fish raw materials without deteriorating organoleptic properties. Goji berries are characterized by low moisture content (12.3%), the presence of pectin substances (2.5%) and fiber (3.6%), high content of potassium (2265 mg/kg), magnesium (1357 mg/kg), calcium (888.1 mg/kg), iron (91.58 mg/kg) and zinc (10.33 mg/kg), which increases the nutritional and functional value of the product.

Experiments have shown that the introduction of 2–6% goji berries ensures uniform distribution of raw materials in the pate, improves taste, aroma, and color characteristics, increases antioxidant potential, and helps extend shelf life. Combining fish raw materials with plant components also allows to increase the content of dietary fiber and biologically active compounds, increase the product's resistance to oxidative processes and create a functional food product that meets modern standards of healthy nutrition.

The introduction of goji berries into fish pâté formulations not only improves the nutritional and functional characteristics of the product but also positively affects its storage stability. The results of physicochemical studies showed that samples containing 2–6% goji berries were characterized by a slower increase in peroxide and acid values during storage compared to the control sample. Due to the presence of natural antioxidants in goji berries, oxidative processes in lipid components were inhibited, which contributed to improved product stability.

Thus, the scientifically substantiated use of goji berries in fish pate recipes allows to obtain a competitive product with high nutritional and biological value, optimal texture and taste characteristics, which confirms the prospects for the introduction of such technologies into the domestic food industry.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper, as well as the published research results, including the financial aspects of conducting the research, obtaining and using its results, as well as any non-financial personal relationships.

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Data availability

The data that support the findings of this study will be made available by the authors on reasonable request.

Use of artificial intelligence statement

The authors confirm that no artificial intelligence technologies were used in the preparation of this work.

Authors' contributions

Nataliia Holembovska: Writing – editing, Conducting research.
Valentyna Israelian: Conducting research.
Natalia Slobodyanyuk: Conceptualisation, Review and Editing.
Anastasiia Ivaniuta: Project administration.
Inna Stetsyuk: Conceptualisation and Project administration.
Inna Kurbatova: Conceptualisation, Review and Editing.
Vasyl Shynkaruk: Conceptualisation, Writing – original draft.
Yaroslav Rudyk: Writing – original draft.

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CHAPTER 7

Innovative approaches to the storage technology of dehydrated meat semi-finished products using natural antioxidants

Liudmyla Kiurcheva
Mykyta Semenov
Serhii Holiachuk

Abstract

The monograph is devoted to the study of innovative approaches to the storage of dehydrated meat semi-finished products from chicken and pork. The combination of convective dehydration modes and treatment with natural antioxidants of meat raw materials during long-term storage was studied.

To preserve the quality and nutritional value of dehydrated raw materials, trans-ferulic acid was used as a natural antioxidant capable of influencing oxidative processes and structural indicators of products. The results of experimental studies on the influence of antioxidant treatment on the dynamics of changes in quality indicators during storage are presented. The optimized parameters of preliminary convective dehydration are considered as a technological possibility of forming a stable product structure to extend the shelf life.

The results of the study can be used by scientists, meat processing industry technologists and food industry specialists in the development of modern technologies for storing dehydrated products and preserving consumer properties.

Keywords

Dehydration, meat semi-finished products, trans-ferulic acid, convective drying, drying kinetics, product quality.

7.1 Introduction

An important task of the modern food industry is the production of long-term semi-finished meat products with high organoleptic indicators and a guaranteed level of safety. Given the high biological value of meat as a source of complete protein,

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essential amino acids, minerals and vitamins, ensuring its stability during storage is one of the priority areas of scientific research. The relevance of this task is enhanced by the need to implement energy-saving and resource-efficient technologies that meet the principles of sustainable development and minimize the ecological burden on the environment.

Among modern methods of preserving meat raw materials, dehydration is considered a promising method for extending the shelf life of products by reducing the mass fraction of moisture and water activity, which helps to slow down microbiological and oxidative processes [1]. Reducing water activity significantly limits the development of microflora and the intensity of enzymatic reactions, ensuring increased microbiological stability of the product during storage.

During the drying process of meat raw materials, complex physicochemical changes occur, namely: protein denaturation, structural transformations of muscle tissue, color changes and lipid oxidation. As a result of such changes, the quality of dehydrated meat products deteriorates, signs of foreign taste and smell appear, and nutritional value decreases, which leads to a reduction in shelf life. In this regard, an urgent scientific and practical task is to improve the technology of dehydration of semi-finished products by optimizing drying modes taking into account moisture removal parameters. A promising direction is the use of natural antioxidant compounds.

Trans-ferulic acid, which belongs to phenolic compounds of plant origin, is characterized by pronounced antioxidant activity and the ability to influence lipid peroxidation processes [2]. Its use in drying technologies for meat raw materials can help preserve organoleptic characteristics and increase the nutritional value of products during storage without the use of synthetic preservatives. However, the use of trans-ferulic acid requires scientific justification and confirmation of safety, compliance with current regulatory requirements for food ingredients

Thus, the use of natural antioxidants in combination with the optimization of drying regimes of meat raw materials is a rather relevant area of research and will have practical significance for the production of dehydrated meat semi-finished products for long-term storage and expanding the range of functional food products.

7.2 Natural antioxidants in meat systems

The key task of the technology for the production of dehydrated meat semi-finished products, storage of meat and meat products is to control the oxidation of lipids and proteins, which determines the degree of stability of food products during long-term storage. Therefore, the main technological problem is the intensification of

oxidative reactions in lipid and protein fractions. Thus, changes in taste, aroma, color and texture are largely associated with the formation of primary and secondary products of lipid peroxidation under the influence of oxygen and thermal factors. Such reactions can lead to the formation of aldehydes, ketones and other volatile compounds, which negatively affect the physicochemical properties and quality characteristics of the product. To reduce the intensity of these processes, natural supplements are used that can inhibit the formation of free radicals and affect the oxidation reaction [3].

Antioxidant substances of plant origin are characterized by the ability to inhibit the oxidation processes of food products, stop spoilage and positively affect the nutritional value of raw materials, therefore they make it possible to use them to replace synthetic additives [4]. Thus, flavonoids, tocopherols, carotenoids, phenolic and other substances include antioxidant compounds capable of neutralizing some forms of oxygen, thus affecting oxidative reactions and processes of lipid and protein oxidation [5].

Phenolic substances of plant origin have the ability to reduce the level of lipid peroxidation in meat raw materials during processing and long-term storage [6], as a result, extending the shelf life of the product while maintaining the qualitative indicators of nutritional value. Thus, the use of plant extracts, such as rosemary extract, grape seed and green tea, demonstrated a positive antioxidant effect on meat raw materials, a decrease in the formation of oxidative products and an improvement in organoleptic properties were observed [7].

Recently, the use of trans-ferulic acid, which is characterized by its ability to affect the charges of the phenolic ring and free radicals, thereby stabilizing oxidative processes in meat raw materials during heat treatment and long-term storage, has attracted particular interest.

When dehydrated, the use of antioxidant substances for the production of semi-finished meat products has a complex meaning: it allows to reduce the negative impact on oxidation processes during the drying process itself; helps reduce the course of these processes during storage of the finished product and has a positive effect on organoleptic indicators and prolonging the shelf life [3]. However, the effectiveness of the use of such antioxidants depends on the method of their introduction, concentration, conditions and parameters of long-term storage, which necessitates further research in this direction.

7.3 Dehydration of raw meat

Dehydration of meat raw materials is one of the preservation methods based on reducing the mass fraction of moisture, as a determining factor in inhibiting the

development of microorganisms. The effect on water activity leads to a decrease in enzymatic processes, inhibition of lipid and protein oxidation processes, which ensures an extension of the shelf life of products [1].

Meat raw materials are a complex multicomponent system in which water has different forms of bonding (adsorbed, osmotic and capillary). Thus, each form is characterized by its own strength and specific effect on the processes of processing and storage of meat products. The removal of moisture from meat raw materials is accompanied by structural changes in the protein-lipid complex, which affects the diffusion processes. The drying process depends on the water-binding capacity and the rate of moisture removal from the depth of the material to the surface. Effective heat and mass transfer in food raw materials is possible only under optimal drying conditions: temperature, air flow rate, product thickness [8].

The choice of technology and drying method is determined by the need for a balance between the speed of dehydration, energy efficiency of the process and the preservation of the organoleptic and structural-mechanical properties of the product. Convective, vacuum, sublimation and combined drying methods are used in the food industry.

Convective drying is based on the removal of moisture by the action of a stream of heated air. At the initial stage of the process, the rate of dehydration is determined by the conditions of external heat and mass transfer. An increase in temperature affects the process of moisture removal, but can cause protein denaturation and acceleration of lipid peroxidation reactions. This is especially critical for meat raw materials, since such processes affect the formation of color, taste and aroma of the product [1].

Vacuum drying removes moisture from raw materials at low temperatures by evaporation under vacuum, which allows dehydration to be carried out at lower temperatures. This process helps to reduce the thermal load on protein structures and preserve pigments and volatile aromatic substances. However, the need to use sealed chambers, vacuum pumps and systems for precise control of process parameters significantly affects production efficiency.

Freeze drying involves pre-freezing the raw material with subsequent removal of ice by sublimation in a vacuum. This method ensures minimal structural damage to tissues and a high ability of the product to rehydrate, which is associated with the formation of a porous structure after the removal of ice crystals. However, the energy intensity and technological complexity of the process limit its use mainly for the production of specialized high-value products.

In the food industry, with the use of innovative technologies, combined approaches are becoming increasingly important, which involve combining different

methods of dehydration or using preliminary preparation of raw materials in order to change their structural and functional properties [9, 10]. Such solutions allow controlling the kinetics of drying and reducing the negative impact of thermal treatment. The combination of optimization of convective drying modes with the use of natural antioxidants aimed at increasing the oxidative stability of the product is especially promising [2].

Thus, the choice of the method and method of drying meat raw materials depends on a comprehensive approach to the physicochemical characteristics of the product, the requirements for its quality and shelf life, as well as the economic feasibility of production. Given the technological availability and the ability to regulate the process parameters, convective drying remains the basic dehydration method for the production of meat semi-finished products with a long shelf life, which can be improved by optimizing the regimes and using antioxidant components.

7.4 Analytical drying models

Mathematical description of the dehydration process is a key stage in the scientific justification of the drying parameters of meat raw materials, as it allows quantitatively characterizing the kinetics of moisture removal, establishing the limiting stages of mass transfer and predicting the duration of the technological cycle. For muscle tissue, which has a complex capillary-porous and protein-lipid structure, internal moisture transfer is limited by diffusion mechanisms, as well as the degree of water binding to the proteins of the myofibrillar complex.

In the case of thin-layer convective dehydration (slice thickness 7 mm, temperature 70°C), the process is usually characterized by the absence of a long period of constant speed and the predominance of the decreasing stage of drying. This indicates diffusion control of the process, which is consistent with the literature data [11].

For such systems, it is advisable to use both phenomenological (based on mass transfer equations) and empirical thin-layer models.

To unify experimental results, the dimensionless parameter Moisture Ratio (MR) is used, which is defined as

$$MR = \frac{M_t - M_e}{M_o - M_e},$$

where M_o – initial moisture content; M_t – moisture content at time t; M_e – equilibrium moisture content.

Under the condition that $M_e \ll M_t$, for thin samples the following simplification is commonly accepted:

$$MR \approx \frac{M_t}{M_o} \text{ or } MR \approx \frac{m_t}{m_o},$$

where m_t – current sample mass; m_o – initial sample mass.

Phenomenological model based on Fick's second law [12, 13]. For a slab of thickness 2*L*, the solution of Fick's second law for unsteady-state diffusion is expressed as

$$MR \approx \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left(-\frac{(2n+1)^2 \pi^2 D_{eff}^t}{4L^2} \right),$$

where D_{eff} – effective moisture diffusivity, m²/s; *t* – drying time, s; *L* – half-thickness of the sample, m.

For long drying times, the series solution can be approximated by its first term

$$MR \approx \frac{8}{\pi^2} \exp\left(-\frac{\pi^2 D_{eff}^t}{4L^2} \right).$$

The estimation of D_{eff} makes it possible to quantitatively compare the intensity of internal mass transfer under different formulation conditions, particularly when trans-ferulic acid is incorporated.

For practical analysis of the drying kinetics of semi-finished meat products, empirical thin-layer models are widely used, as they provide high accuracy in approximating experimental drying curves [14].

Model Page.

$$MR = \exp\left(-kt^n\right),$$

where *k* – drying rate constant; *n* – process nonlinearity exponent.

The Page model is a modification of the simple exponential relationship and adequately describes the diffusion-controlled stage of dehydration of biological materials, including meat raw materials [15].

Henderson-Pabis model.

$$MR = A \exp\left(-kt\right),$$

where *A* – empirical constant; *k* – drying rate coefficient [16].

A comparative analysis of the Page and Henderson–Pabis models make it possible to determine the degree of agreement with experimental data and to establish the influence of formulation factors on process kinetics [17].

Criteria for evaluating model adequacy [18].

The goodness of fit was assessed using the coefficient of determination (R^2)

$$R^2 = 1 - \frac{\sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pred,i}\right)^2}{\sum_{i=1}^{N}\left(MR_{exp,i} - \overline{MR}_{\exp}\right)^2},$$

and the root mean square error (RMSE)

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pred,i}\right)^2},$$

where N – number of experimental data points.

For food systems, the values of $R^2 > 0.90$ are considered acceptable, while $R^2 > 0.95$ indicates a high correspondence of the model to the experimental data.

Within the framework of this work, the model was applied to describe the kinetics of convective dehydration of meat semi-finished products at a temperature of 70°C and a slice thickness of 7 mm. Special attention was paid to assessing the effect of the introduction of trans-ferulic acid on the parameters k, n, and D_{eff}.

It is assumed that the antioxidant can affect the structural and mechanical properties of muscle tissue, change the degree of moisture binding and, accordingly, the kinetics of internal mass transfer. Comparison of the values of the effective diffusion coefficient and activation energy allows to quantitatively assess the structural changes of the system under the action of the functional additive.

Thus, the combination of a phenomenological approach (based on Fick's second law) and empirical thin-layer models provides a comprehensive interpretation of the dehydration process and creates a scientific basis for optimizing technological regimes.

7.5 Experimental implementation of convective dehydration of meat raw materials using trans-ferulic acid

The object of the study is the process of convective dehydration of meat raw materials under the conditions of the prescription introduction of a natural antioxidant compound – trans-ferulic acid (FA). The subject of the study is the kinetic patterns of moisture removal and quality indicators of dehydrated meat semi-finished products depending on the type of raw material and the presence of an antioxidant additive.

The purpose of developing the recipe is to increase the antioxidant properties of dehydrated semi-finished meat products (pork meat, chicken meat) by treating them with trans-ferulic acid (FA) in a minimum concentration to ensure an effect on oxidative processes without negative changes in the organoleptic and safety indicators of the product.

The materials of the study: muscle tissue of chicken meat (breast fillet without skin; initial mass fraction of moisture 74–76%); muscle tissue of pork meat (tenderloin; initial mass fraction of moisture 72–74%).

Trans-ferulic acid with a mass fraction of the main substance ≥ 98% was used as a functional additive. In order to ensure uniform distribution in meat samples, the antioxidant additive was previously dissolved in food alcohol (96%).

The marinade composition (per 100 g of raw material) included:

– sodium chloride – 1.2%;

– dried paprika – 0.5%;

– ground black pepper – 0.3%;

– trans-ferulic acid – 0.1% (in the form of an ethanol solution).

This formulation allows to simulate the conditions of production of dehydrated meat semi-finished products with a spicy-salt profile and at the same time minimize the influence of extraneous ingredients on the drying kinetics.

The selected concentration of FA (0.1%) is technologically appropriate considering the combination of antioxidant efficiency and sensory properties. Lowering the concentration may be insufficient to inhibit lipid peroxidation, while increasing it can potentially affect the flavor profile of the product.

The functional role of the marinade components: salt for osmotic effect and flavor formation; paprika acts as a natural colorant and a source of phenolic compounds; black pepper for aroma formation; trans-ferulic acid serves as the antioxidant aimed at inhibiting lipid oxidation chain reactions and stabilizing the pigment system. Thus, the prescription composition forms a multicomponent antioxidant background, where FA performs a dominant stabilizing function.

The study was conducted according to a two-factor experimental design considering:

Factor A – type of meat raw material:

A_1 – chicken meat;

A_2 – pork meat.

Factor B – formulation composition:

B_1 – control sample (without FA);

B_2 – experimental sample (FA at a dose of 0.1 g per 100 g of raw material, i.e., 0.1%).

In total, four experimental variants were formed:

A_1B_1 – chicken meat, control;

A_1B_2 – chicken meat + FA;

A_2B_1 – pork meat, control;

A_2B_2 – pork meat + FA.

The introduction of trans-ferulic acid was carried out by its preliminary dissolution (0.1 g FA in 10 ml of ethanol 96%) with subsequent introduction into the marinade and thorough mixing. The prepared samples were marinated for 60 min at a temperature of 4–6°C with periodic mixing to ensure the diffusion of salt, spices and antioxidant compounds into the thickness of the muscle tissue. After the completion of marinating, the surface of the samples was dried with filter paper to remove excess moisture.

Drying was performed in a laboratory dehydrator with forced air circulation. The process parameters were as follows: drying agent temperature – 70°C; slice thickness – 7 mm; air velocity – approximately 2 m/s; drying duration – 8 hours.

The choice of temperature 70°C is due to the need to ensure sufficient moisture removal intensity while preserving the organoleptic properties of the raw material and limiting thermal denaturation of proteins. The thickness of 7 mm corresponds to the conditions of a thin layer, which is methodologically correct for the further application of analytical models of drying kinetics.

Before the start of the process, the samples were weighed to determine the initial mass (m_o). Control weightings were carried out after 2, 4, 6, and 8 hours (t_o, t_1, t_2, t_3, t_4). After the dehydration was completed, the samples were cooled to 25°C, packed in sealed polymer bags and stored at 20 ± 2°C.

The results obtained (**Table 7.1**) indicate that under the same dehydration conditions, the introduction of trans-ferulic acid at a dose of 0.1% does not cause statistically significant differences in total mass losses after 8 h of drying. This gives grounds to believe that the antioxidant additive does not have a significant effect on the overall degree of dehydration (**Fig. 7.1**), and possible differences are mainly associated with changes in the kinetics of the process at individual stages.

Table 7.1 Moisture loss during convective drying (70°C, $n = 1$)

Sample variant	Mass, g					Mass loss, %
	(0 h)	(2 h)	(4 h)	(6 h)	(8 h)	
A_1B_1	71.9	52.7	41.2	32.9	25.8	64.1
A_1B_2	73.8	54.3	42.0	33.8	26.6	64.0
A_2B_1	74.2	57.8	46.9	40.1	32.8	55.8
A_2B_2	75.1	58.9	47.7	40.6	33.5	55.4

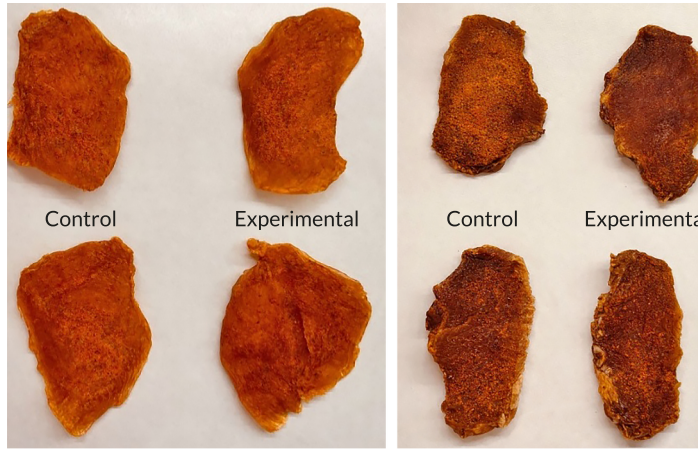

Fig. 7.1 Appearance of samples after drying (control and experimental)

The dimensionless moisture content was used to construct the kinetic curves (Moisture Ratio, MR)

$$MR = \frac{m_t}{m_o}.$$

Further mathematical processing of the experimental data was carried out by approximating the Page and Henderson-Pabis models, as well as determining the effective diffusion coefficient based on Fick's equation. The generalized parameters are presented in **Table 7.2**.

The Page model demonstrates higher approximation accuracy for all sample variants the coefficient of determination R^2 ranges from 0.9993 to 0.9998; the RMSE values do not exceed 0.0053.

Table 7.2 Approximation results

Sample variant	Henderson-Pabis A	Henderson-Pabis k, h^{-1}	R^2	RMSE	Page k	Page n	R^2	RMSE
A_1B_1	0.970	0.1261	0.9940	0.0175	0.1708	0.8560	0.9998	0.0029
A_1B_2	0.969	0.1258	0.9937	0.0179	0.1692	0.8612	0.9998	0.0028
A_2B_1	0.972	0.0999	0.9920	0.0175	0.1398	0.8427	0.9993	0.0053
A_2B_2	0.974	0.0993	0.9931	0.0162	0.1351	0.8575	0.9995	0.0044

For the Henderson-Pabis model, R^2 falls within the range of 0.9920–0.9940, which also indicates satisfactory agreement, though inferior to the Page model. The effect of FA on dehydration kinetics is minimal.

For pork samples, the drying rate constant k(H–P) values are: control – 0.0999 h^{-1}; FA – 0.0993 h^{-1}.

A similar trend is observed for chicken fillet: control – 0.1261 h^{-1}; FA – 0.1258 h^{-1}.

The difference does not exceed 1%, indicating no significant influence of the antioxidant on mass transfer intensity at 70°C.

The type of raw material is the determining factor in drying kinetics.

Drying rate constants for chicken fillet are higher than for pork. This can be explained by the lower content of intramuscular fat, lower tissue density, and higher proportion of free moisture.

The graphical representation of the $MR(t)$ relationships (**Fig. 7.2**, **7.3**) confirms the gradual decrease in relative moisture content, the absence of a constant-rate drying period, and the predominance of a diffusion-controlled mass transfer mechanism.

The analysis showed that the Page model more accurately approximates the experimental data (higher R^2 values and lower RMSE) compared to the Henderson-Pabis model. The addition of trans-ferulic acid (0.1%) did not affect the shape of the kinetic curves, indicating that it had no significant effect on the dehydration rate. Chicken meat is characterized by higher drying rate coefficients, indicating more intensive dehydration compared to pork.

The curves for the control and experimental samples are practically superimposed, which confirms the technological compatibility of FA with the convective dehydration process.

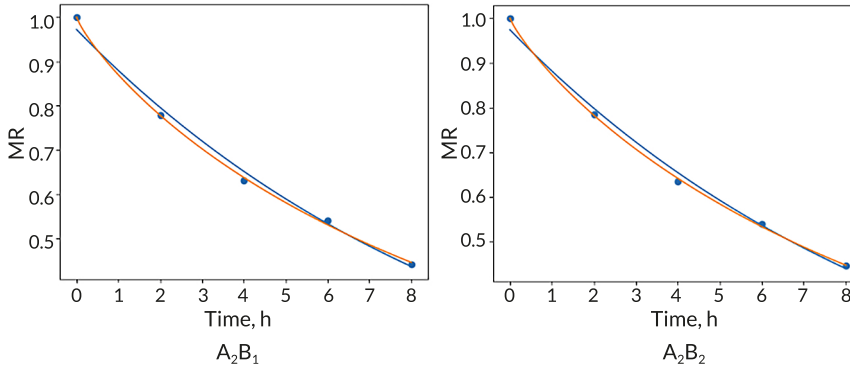

Fig. 7.2 Dehydration kinetics of pork

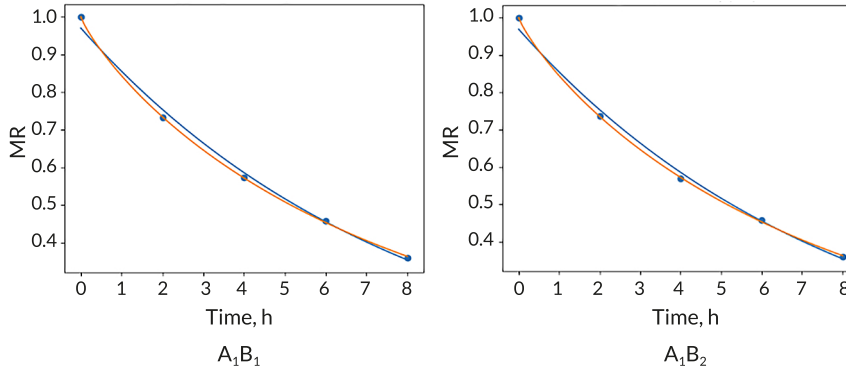

Fig. 7.3 Dehydration kinetics of chicken meat

A comparison of the model parameters allows the conclusion that the incorporation of FA at a concentration of 0.1% does not alter the shape of the kinetic curves; the parameters *k* and *n* change only slightly, and the overall degree of dehydration after 8 hours remains the same. Therefore, the antioxidant does not affect the heat and mass transfer characteristics of the system and does not require adjustment of the drying modes.

Organoleptic evaluation was carried out after cooling the samples to 25°C. The control and experimental samples were characterized by uniform dehydration, no signs of burning, elastic texture, typical aroma for the corresponding type of meat. The introduction of FA did not cause the appearance of foreign odor or taste. The color of the experimental samples remained stable and characteristic of thermally processed meat raw materials

7.6 Conclusions

A comprehensive analysis of experimental data showed that the dehydration kinetics for all samples is characterized by a predominantly decreasing drying rate, which confirms the diffusion mechanism of internal mass transfer. The process is adequately described by empirical models, with the Page model providing the highest accuracy of approximation of experimental data.

Comparison of control and experimental samples showed that the introduction of trans-ferulic acid at a concentration of 0.1% does not violate the heat and mass transfer conditions of the process, does not significantly affect the rate of moisture

removal and does not require adjustment of drying modes at 70°C. At the same time, a tendency to a slight change in kinetic parameters (rate constant *k* and nonlinearity index *n*) was recorded, which may be associated with the structural features of the protein-water matrix and the degree of moisture binding.

It was found that chicken fillet is dehydrated more intensively compared to pork, which is due to morphological and compositional differences in the raw materials.

Therefore, the use of trans-ferulic acid at a dose of 0.1% is technologically justified and compatible with the convective drying process, does not worsen organoleptic characteristics and creates prerequisites for increasing product stability. At the same time, the results indicate the need for further statistical verification and investigation of oxidative stability to assess the overall effectiveness of the additive.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper, as well as the published research results, including the financial aspects of conducting the research, obtaining and using its results, as well as any non-financial personal relationships.

Financing

The study was performed without financial support.

Data availability

The data that support the findings of this study will be made available by the authors on reasonable request.

Use of artificial intelligence statement

The authors used the AI assistant Perplexity (Grok 4.1, Perplexity AI) for translation and literature source selection. The authors bear full responsibility for the final manuscript. Generative AI tools are not credited and are not responsible for the final results.

Authors' contributions

Liudmyla Kiurcheva: Supervision, Conceptualization, Methodology, Writing – original draft, Investigation, Project administration.

Mykyta Semenov: Conceptualization, Methodology, Writing – original draft, Formal analysis, Investigation.

Serhii Holiachuk: Writing – original draft, Visualization, Formal analysis, Validation.

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CHAPTER 8

Development of sauce technology from fermented plant-based materials for the food industry and HoReCa

Marina Serdyuk
Valentyna Bandura
Tetiana Kolisnychenko
Karina Palamarek
Olha Romanovska
Tetiana Brykova
Tetiana Marusiak
Anastasia Parashchuk

Abstract

The research is aimed at developing a sauce technology from fermented plant-based materials of various origins for use in the food industry and the HoReCa segment. The objects of the research were fermented legume, grain, pseudo-grain, and vegetable crops, as well as sauces prepared on their basis according to three experimental formulations. The control was a sauce prepared using traditional technology based on boiled chickpeas. Lactic acid bacteria of the species *Lactiplantibacillus plantarum* were used as biocatalysts during the fermentation of all types of plant substrates. An assessment of physicochemical changes in plant-based materials before and after fermentation was carried out with an emphasis on determining the pH, redox potential, titrated acidity, content of proteins, fats, total carbohydrates, and sugars. A significant decrease in pH was established, indicating the active accumulation of organic acids. At the same time, a shift in the redox potential towards a reducing environment was recorded, which is typical for anaerobic fermentation conditions, especially in samples with a high sugar content. A substantial decrease in the total content of carbohydrates, in particular sugars, was observed due to their utilization by microorganisms. The changes obtained indicate an improvement in the functional properties of plant-based materials after fermentation. The energy value of ready-made sauces based on the selected formulations was determined. It was established that samples from fermented plant-based materials have a lower energy

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value compared to the control, which is associated with the biochemical degradation of part of the sugars and partial cleavage of organic compounds under the effect of the enzymatic activity of microorganisms. Organoleptic evaluation showed a clear formation of new flavor profiles in sauces from fermented plant-based materials. All variants demonstrated a highly stable and homogeneous consistency. Based on the results of experimental studies, a functional sauce technology from fermented plant-based materials for the food industry and HoReCa was developed. According to the results of economic calculations, all samples of sauces prepared according to the experimental formulation had a higher level of profitability compared to the control. The results obtained confirm the feasibility of producing sauces using the developed technology based on the use of fermented plant-based materials in the food industry and the HoReCa segment, both from the standpoint of technological and functional advantages and economic efficiency.

Keywords

Sauces, fermentation, chickpea, carrot, oat, lentil, quinoa, tomato, zucchini, barley, green pea.

8.1 Introduction

Modern trends in the development of innovative technologies for the food industry and the HoReCa segment are aimed at constantly increasing the requirements for organoleptic indicators, nutritional and biological value, as well as functional properties of the product. In modern conditions, the global market for food products with new qualities that contribute to improving human health and well-being is rapidly developing. Consumer demand for high-quality natural products that do not contain synthetic food additives, but instead have improved functional properties due to the introduction of natural enriching ingredients, is increasing [1]. According to the leading nutritionists worldwide, sauces play the most important role in human nutrition. They provide unique taste accents, enrich the aroma, increase nutritional and biological value, and, due to the stimulation of the digestive glands, improve food digestibility. Sauces ensure the juiciness of dishes, increase calorie content, and diversify the diet, making products with a bland taste more attractive and in high demand among consumers [2].

However, traditional technologies for making sauces involve deep heat treatment of the raw materials, which significantly reduces the biological value. Their formulations contain increased concentrations of salt, sugar, and fat. Synthetic preservatives are added to sauces to increase their shelf life, and structure stabilizers are added

to ensure rheological properties. This approach does not align with modern theories of healthy eating, which have gained particular popularity all over the world [2, 3].

Fermentation is one of the most promising ways to increase the functional properties of sauces based on natural plant-based materials. Thanks to the use of beneficial microorganisms, plant-based materials are transformed into valuable food products during fermentation. Such products have an extended shelf life, improved consistency, a unique taste and aroma, as well as an increased nutritional value created by probiotics [3]. Modern scientific literature presents the results of studies that explain the mechanisms of fermentation processes in food systems [4, 5]; however, the issue of using fermented plant-based materials for the preparation of sauces remains almost unexplored.

Another problem is the development of a universal technological process adapted to the conditions of modern restaurants, catering services, and craft food production. For modern establishments of the HoReCa segment, a special requirement is a short production cycle and high quality of the finished product, which must be standardized. For food industry enterprises, in addition to the above, the reproducibility of technological parameters, scalability, and economic indicators of production are no less important.

From this point of view, there is a lack of a scientifically substantiated technology for the production of sauces for the food industry and the HoReCa segment, which would meet all modern trends and ensure the production of sauces with improved functional and sensory properties, high production, and economic attractiveness. Solving this problem requires a detailed experimental study of the mechanisms influencing fermentation on the properties of plant substrates, as well as scientifically based modeling of formulations of sauces from fermented plant-based materials.

8.2 Scientific and theoretical fundamentals for the development of functional sauces from fermented plant-based materials

Modern prospects for the development of the food product range are aimed at meeting the specific needs of different population groups. In this regard, dishes for vegans and vegetarians, low-sugar, gluten-free, lactose-free, and high-protein complete diets are becoming increasingly popular. Such trends determine the expansion of the specific line of sauces based on natural plant-based materials. They will contribute to the enrichment of the diet with valuable biologically active substances, in particular vitamins, phenolic substances, natural dietary fibers, and minerals [4, 6].

The most promising plant-based materials for the production of sauces are legume, grain, pseudo-grain and vegetable crops. The complementarity of the functional and technological properties of these crops and a possible synergistic effect of their combination in the formulations are of special scientific interest.

Legume crops are traditionally considered the most complete source of vegetable protein, as well as soluble and insoluble dietary fiber. Protein of legume crops is complete and characterized by a high content of amino acids. It is very well absorbed by the human body. In addition, protein fractions of legumes exhibit high moisture-retaining and fat-retaining capacity, which ensures the ability of raw materials to swell. Dietary fiber also has pronounced structure-forming properties. Soluble dietary fiber, such as pectin and hemicellulose, increases the viscosity of food systems, insoluble fiber forms the structural basis of the product and determines its consistency. Thus, dietary fiber performs the functions of natural stabilizers of dispersed food systems. It should also be noted that soluble dietary fiber is directly involved in the processes of regulating blood glucose levels and lipid metabolism [7]. Therefore, the use of legume crops will contribute to the formation of a stable, homogeneous consistency of sauces without the use of synthetic stabilizers.

A well-known drawback of legumes is the presence of antinutrients that reduce the bioavailability of essential minerals. They contain phytic acid, tannins, and raffinose oligosaccharides [8, 9]. Phytic acid has the ability to create chelate complexes with calcium, iron, and zinc, which complicates their absorption. Raffinose and stachyose induce gas formation in the intestine during fermentation. In view of this, when developing the processing technology, it is advisable to provide methods of pre-treatment of legume crops that will help reduce the content of these substances or convert them into more accessible forms.

Cereals and pseudocereals can be considered as a functional supplement to the protein fraction of legumes. The correct combination of these crops ensures the formation of a complementary amino acid composition, as well as the supply of useful complex carbohydrates, starch, β-glucans, phenolic substances, and vitamins. In the presence of moisture, polysaccharides of cereals and pseudocereals are intensively hydrated and form viscous colloidal systems. This has a positive effect on the consistency and rheological properties of sauce systems. The most useful high-molecular soluble polysaccharides of cereals are β-glucans. They form stable hydrocolloid structures, significantly increase the moisture-binding and moisture-retaining capacity of the product, and strengthen its structural stability. In addition, β-glucans are characterized by a high ability to reduce blood cholesterol levels [10–12]. As for the phenolic substances of cereals and pseudocereals, most of them are in a bound form, but they can be considered as a promising source of substances with antioxidant activity [13].

Plant-based materials determine organoleptic properties and micronutrient composition of sauces. The high content of β-carotene, lycopene, polyphenols, ascorbic acid, and mineral elements, compared to other plant-based materials, forms the functional properties and attractive natural color of the product [12, 14]. Pectic substances, starch, cellulose, hemicellulose, and other structural polysaccharides of vegetables take an active part in creating a stable consistency, as they are able to form gels and ensure the stability of emulsions.

Microbiological fermentation contributes to a more complete use of the functional properties of plant-based materials in the production of food products [15]. During fermentation, deep transformations of proteins, carbohydrates, and phenolic substances of plant-based materials occur. Active forms of microorganisms transform complex compounds into simpler and more accessible forms, reduce the concentration of antinutrients, and stimulate the synthesis of biologically active substances [16, 17].

Thus, the research results [18] showed that during the fermentation of legume crops by lactic acid bacteria of the genus *Lactobacillus*, hydrolysis of phytic acid occurs, and the content of raffinose oligosaccharides decreases [18]. During the fermentation of grain raw materials, the enzyme systems of both grains and microorganisms that take a direct part in the process are activated. This results in partial cleavage of polysaccharides and the release of bound phenolic compounds, increasing antioxidant properties [19]. During the fermentation of plant-based materials, the main result is the formation of organic acids – primarily lactic and acetic. This is accompanied by a decrease in pH, inhibition of the development of pathogenic microorganisms, extension of the shelf life of the product, and improvement of its functional properties [20, 21].

In this regard, lactic acid fermentation can be an effective technological method for improving the functional properties of natural sauces based on plant-based materials. Lactic acid bacteria are the main biocatalysts of fermentation processes. In the process of metabolic activity during growth and development, they convert sugars into lactic acid. This provides a decrease in pH, which inhibits the development of pathogenic microorganisms and extends the shelf life of products [22].

The products of the metabolic activity of lactic acid bacteria during fermentation are not only organic acids. As a result of the synthesis, volatile aromatic compounds are also formed. They ensure the formation of a complex, pleasant aroma of the product [23]. Depending on the ingredients of the formulation, pleasant creamy, spicy, sour, or umami tastes can dominate in the finished fermented sauces. This is crucially important, since the taste and aroma profile is the most important indicator of the organoleptic assessment of sauce products.

Exopolysaccharides are the metabolic products of certain strains of lactic acid bacteria. They are high-molecular compounds that are responsible for the density of the product, improve moisture-binding and moisture-retaining properties, and prevent emulsion delamination. This naturally ensures a stable, homogeneous consistency of sauces [24, 25].

The fermentation also results in a change in the composition of proteins and phenolic substances. Peptides and free amino acids are formed through proteolysis. They take an active part in the formation of the taste and aroma profile of food systems, and some even exhibit antioxidant activity. Along with this, the availability of micronutrients increases, and the synthesis of B vitamins also occurs [26]. This significantly increases the functional properties of ready-made sauces.

The efficiency of microbiological fermentation is determined by a set of technological parameters. The most important of them are the temperature, process duration, physicochemical properties of the substrate, and the type of strain of microorganisms [27]. By controlling and changing technological parameters, the intensity of metabolic development of microorganisms can be regulated, and the properties of finished sauces can be optimized.

Controlled fermentation using pure starter cultures of lactic acid bacteria provides predictable acidity, reproducibility of organoleptic properties, and stability of quality indicators compared to spontaneous fermentation [28]. This is of fundamental importance for the food industry and the HoReCa segment, where standardization of formulations, stability of indicators, and the possibility of scaling production are necessary.

Thus, the combination of complementary plant-based materials with controlled lactic acid fermentation forms a holistic technological concept for creating functional sauces. This approach allows for a simultaneous increase in biological value, ensures structural stability, forms a complex natural sensory profile, and adapts the product to the requirements of the food industry and the HoReCa segment. The set of the presented modern scientific data confirms the scientific validity of this direction and its prospects for expanding the range of new generation sauces.

8.3 Modeling of formulations of functional sauces from fermented plant-based materials

The aim of the research was to develop a technology for the production of functional sauces from fermented plant-based materials of various natures for use in the food industry and the HoReCa segment.

To achieve this aim, the following tasks were set: to investigate the kinetics of physicochemical changes in plant-based materials during the fermentation process; to analyze the content of mass fractions of macronutrients in experimental samples of sauces and determine their energy value; to conduct an organoleptic assessment of sauces from fermented plant-based materials and compare it with the control option; to develop a technology for the production of sauces from fermented plant-based materials taking into account the requirements of the food industry and HoReCa, determining rational technological parameters and stages of the process.

Three variants of formulations from fermented plant-based materials were developed for the research (**Table 8.1**).

A traditional sauce based on boiled, unfermented chickpeas, olive oil, lemon juice, garlic, and spices was used as a control (**Table 8.1**). Fermented plant-based materials were not included in the formulation of the control sample, which allowed to carry out an objective assessment of the effect of fermentation on the organoleptic properties, physicochemical parameters, content of basic macronutrients, and energy value of experimental sauce samples.

The main raw materials selected were legumes, including chickpeas, green peas, lentil; vegetables, including carrots, zucchini, tomatoes; cereals, including oats, barley; and a pseudocereal crop, quinoa. All industrial raw materials were purchased from the SILPO retail chain. Legumes and cereals were produced by "Arnika Organic" Agroindustrial Group, and vegetable crops were produced by "Ovochevy Svit" LLC, Ukraine.

Four experimental sauce samples were prepared under laboratory conditions, according to the given formulation (**Table 8.1**), each in five replicates.

The manufacturing process was carried out using the equipment of the Educational and Scientific Laboratory of Food Technology of the National University of Life and Environmental Sciences of Ukraine (Kyiv), in particular a washing bath, a grinder, an incubation chamber with adjustable temperature and humidity parameters, a homogenizer with adjustable rotations, a thermostatic cabinet for processing liquid components, sterile containers for fermentation and a pH meter for monitoring acidity during the fermentation process.

To prepare the control sample, chickpeas were pre-filled with drinking water in a ratio of 1:3 and kept for 12 hours. After the end of the holding period, the swollen grains were separated from the water and washed 2 more times. Such careful preparation of chickpea grains contributes to the leaching of a significant proportion of antinutrients. Next, the chickpeas had been boiling until fully cooked at a temperature of 100°C for 45 minutes. After cooking, they were cooled to a temperature of 18 ± 2°C. The sauce was prepared by mixing all the recipe ingredients (**Table 8.1**) in a laboratory homogenizer. The rotation speed of the homogenizer shaft was

3000 rpm, and the mixing duration was 3 min. As a result, a sauce of a homogeneous pasty consistency was obtained. The finished control samples were packed in sterile, sealed plastic containers, labeled, and stored in a refrigerator at a temperature of 4 ± 1°C until experimental studies were conducted, but no longer than for 5 days.

Table 8.1 Formulations of sauces from fermented plant-based materials

Control formulation (Cf)		Fermented Chickpea – Carrot – Oat (FCCO)	
Ingredients	**Mass, g**	**Ingredients**	**Mass, g**
Boiled chickpea	60.0	Fermented chickpea	30.0
Water	25.0	Fermented carrot	18.0
Olive oil	8.0	Fermented oats	12.0
Table salt	1.2	Vegetable broth	25.0
Lemon juice	2.0	Olive oil	8.0
Fresh garlic	1.0	Table salt	1.2
Smoked paprika	0.8	Lemon juice	2.0
Cumin	0.6	Roasted garlic	1.0
Turmeric	0.5	Smoked paprika	0.8
Black pepper	0.9	Cumin	0.6
–	–	Turmeric	0.5
–	–	Black pepper	0.9
Total	**100**	**Total**	**100**
Fermented Green Pea – Zucchini – Barley (FGZB)		**Fermented Tomato – Lentil – Quinoa (FTLQ)**	
Ingredients	**Mass, g**	**Ingredients**	**Mass, g**
Fermented green peas	23.2	Fermented tomato	31.0
Fermented zucchini	20.3	Fermented lentils	18.6
Fermented barley	14.5	Fermented quinoa	12.4
Vegetable broth	27.7	Vegetable broth	22.2
Pumpkin seed oil	7.0	Red basil oil	7.0
Table salt	1.0	Table salt	1.2
Apple vinegar	2.0	Balsamic vinegar	2.0
Fermented garlic	1.0	Roasted garlic	1.0
Ground coriander	1.0	Smoked paprika	0.8
Dried mint	0.5	Chili pepper	0.6
Fresh dill	1.0	Cloves	0.2
Fresh green onion	0.8	Inulin	3.0
Total	**100**	**Total**	**100**

Before preparing experimental samples of sauces, fermentation of plant-based materials was carried out. Plant-based materials were ground in a universal laboratory blender to particles ≤ 5 mm in size. Chickpeas, red lentils, oats, and quinoa were moistened to 68 ± 2%. After that, fermentation was carried out.

Lactic acid bacteria of the species *Lactiplantibacillus plantarum* were used as biocatalysts during the fermentation of all types of plant substrates at a concentration of 10^6–10^7 CFU/g. Fermentation was carried out in a thermostat at 30 ± 1°C. The process was stopped after 24 hours of fermentation and reaching the substrate pH at the level of 4.1–4.3 by cooling to 1 ± 1°C. Creating the same fermentation conditions for all experimental samples of plant-based materials eliminated them as an influencing factor and ensured a correct scientific comparison of changes in the properties of sauces under the influence of the recipe ingredients.

Fermented plant ingredients were stored in the refrigerator at 1 ± 1°C in airtight containers for further experimental research and preparation of sauces, but no longer than 7 days.

To prepare experimental samples of sauces, a certain amount of recipe ingredients was mixed in a laboratory homogenizer at a shaft rotation frequency of 3000 rpm for 3–5 minutes until a homogeneous consistency was formed, packed in sterile airtight plastic containers, labeled, cooled, and stored at 4 ± 1°C until experimental research was conducted, but no longer than for 5 days.

Organoleptic and physicochemical indicators of the fermented plant-based materials and experimental samples of sauces were evaluated, the content of essential nutrients was determined, and the energy value was calculated.

Organoleptic characteristics were evaluated by the method of profile analysis with the involvement of a tasting commission using a descriptor approach. Appearance, color, aroma, consistency, and flavor profile were evaluated.

Physicochemical parameters of the raw materials before and after fermentation were determined by generally accepted methods. Active acidity (pH) was measured by the potentiometric method using a calibrated pH meter at a temperature of 20 ± 2°C. Redox potential (ROP) was determined electrochemically using a platinum electrode [29]. Titrated acidity was determined by the method of neutralization titration with 0.1 N NaOH solution, with the results expressed in terms of lactic acid [30].

The mass fraction of sugars was determined by the ferricyanide method. This analytical method is based on the ability of reducing sugars to convert potassium ferricyanide ($K_3[Fe(CN)_6]$) to potassium ferricyanide ($K_4[Fe(CN)_6]$) under alkaline conditions. The reaction occurs by reducing ferricyanide ions with monosaccharides present in the sample. Methylene blue was used as a redox indicator. During the reaction, the characteristic blue color gradually disappears as the ferricyanide

is reduced, resulting in a color change to a pale yellow or almost colorless solution, indicating the titration end point [31].

Protein content was determined by the Kjeldahl method using a conversion factor of 6.25. Fat mass fraction was determined by extraction in a Soxhlet apparatus. Total carbohydrates were determined by laboratory method after acid hydrolysis of samples with subsequent quantitative determination of the carbohydrate fraction by the spectrophotometric method. Dietary fiber mass fraction was determined by the gravimetric method with enzymatic treatment [29]. Energy value was calculated based on the obtained data on the content of proteins, fats, and carbohydrates using generally accepted conversion factors [32].

All determinations were performed in at least five replicates. The results were processed using mathematical statistics methods, using mean values and standard deviation.

8.4 Changes in the physicochemical parameters of plant-based materials under the influence of fermentation

The most important condition for ensuring the predicted quality of functional sauces and the controllability of the technological process of their production is scientific monitoring of the physicochemical parameters of raw materials at the stage of lactic acid fermentation. The degree of metabolic activity of lactic acid bacteria is characterized by changes in the hydrogen index (pH), redox potential (Eh), titrated acidity (TA), and basic macronutrients.

The active acidity of the environment, which is characterized by the value of the hydrogen index, is of primary importance for ensuring the metabolic activity of lactic acid bacteria during fermentation. Achieving the required pH level ensures the formation of product taste qualities and extends its shelf life.

The results of experimental studies, presented in **Fig. 8.1**, show that the initial pH values mainly ranged within 6.11…6.44 depending on the type of plant-based materials, with the exception of tomatoes with an initial pH of 4.32, which are typical values for fresh plant substrates. After fermentation, the pH decreased to the range of 3.78…4.56 depending on the type of raw materials.

After 24 hours of fermentation, the pH of most plant substrates, in particular carrots, zucchini, green peas, barley, decreased to the range of 4.1…4.3, which contributed to the formation of excellent organoleptic indicators and high preservation.

In dry legume extracts (chickpeas, lentils) and quinoa, the value of the hydrogen index after 24 hours of fermentation exceeded the limit of 4.3, but its decrease was

significant and was within 1.7–1.8 units. Such dynamics are associated with the peculiarity of the chemical composition of these crops, in particular, a higher content of proteins, which provides an increase in buffer capacity.

During the fermentation of tomatoes, the decrease in the hydrogen index pH was minimal and amounted to only 0.54 units. However, the low initial value and its corresponding decrease characterize a sufficient level of fermentation and contribute to the formation of a stable acidic environment.

It should be noted that, depending on the type of substrate, the decrease in pH ranged from 1.71 to 2.18 units. Such a change in the hydrogen value fully corresponds to the typical dynamics of lactic acid fermentation of plant substrates. Achieving a pH value ranging from 4.1 to 4.3 has high technological significance. At the same time, the development of most types of pathogenic microflora is inhibited, and prerequisites for the production of sauces with an extended shelf life are formed.

The metabolic activity of lactic acid bacteria during fermentation is affected not only by a change in the pH value. Under their action, profound changes in biochemical substrates occur, which significantly affect the redox balance of the environment (**Fig. 8.2**).

Before the start of fermentation, almost all plant substrates were characterized by a negative redox potential with fluctuations ranging within –40…–65 mV.

This may be due to the high content of proteins and amino acids, phenolic substances, and active enzymes. The only exception was tomatoes, for which the ORP value was positive and amounted to 120 mV. This is due to higher natural acidity, low protein content, and, accordingly, lower buffer capacity. It should also be noted that tomato tissues are characterized by a higher proportion of dissolved oxygen, as a result of which a more oxidative environment was formed, compared to other crops.

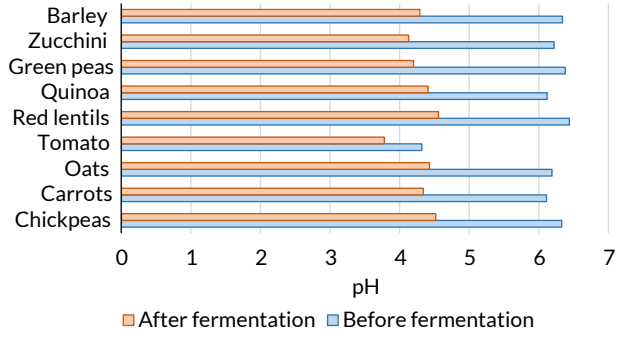

Fig. 8.1 Changes in pH of plant materials during fermentation

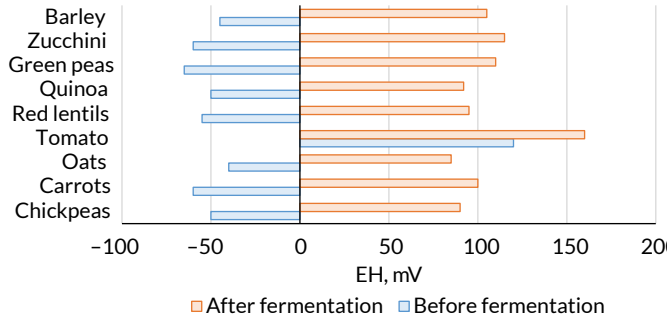

Fig. 8.2 Changes in the redox potential (Eh) of plant-based materials during fermentation, mV

After fermentation, the redox potential increased in all plant substrates without exception to the limits of +85...+160 mV. This increase is fully correlated with the increase in pH and indicates changes in the physicochemical parameters of plant substrates and the formation of a stable acidic environment, which will provide unique organoleptic indicators of future sauces and contribute to the extension of their shelf life.

Titrated acidity (TA) is considered the main indicator characterizing the intensity of lactic acid fermentation. It enables the estimation of the total amount of acids in the fermented system. It is generally known that the metabolic activity of lactic acid bacteria may result in the accumulation of secondary metabolites of fermentation, i.e. organic acids. The main acid formed during the fermentation of plant substrates is lactic acid. The formation of acetic acid is also possible. The level of TA growth characterizes the intensity of their accumulation.

The results of experimental studies (**Fig. 8.3**) indicate a statistically significant increase in titrated acidity (TA) in all plant substrates after lactic acid fermentation.

The initial content of titrated acids was typical for fresh plant-based materials and ranged from 0.035 to 0.16%. This indicator was minimal in grain and pseudo-grain substrates, and it was maximal in tomatoes.

After fermentation, the titrated acidity increased by 5–9 times depending on the nature of the plant substrate. The maximum growth of this indicator was established for green peas, zucchini, and tomatoes, which indicates a high metabolic activity of lactic acid microflora in these plant substrates. The increase was somewhat lower for barley, quinoa, and carrots. The different dynamics of this indicator may be associated with the peculiarities of the chemical nature of plant substrates, as well as the varying degree and speed of involvement of polysaccharide

complexes in the fermentation processes. The obtained values of titrated acidity after fermentation indicate the accumulation of a sufficient amount of organic acids in all plant substrates to ensure the necessary taste characteristics of future sauces.

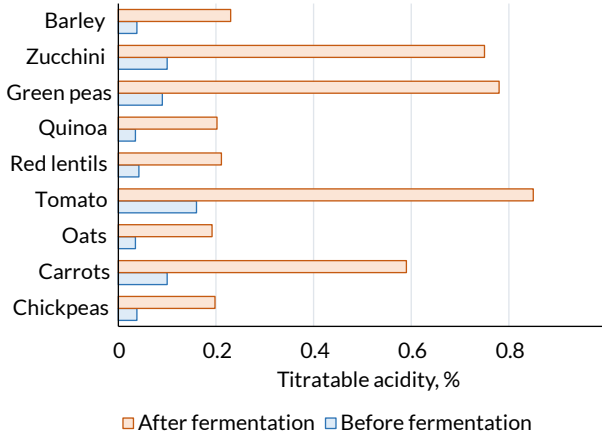

Fig. 8.3 Titrated acidity (TA) of plant substrates after fermentation, %

Another criterion that allows for establishing the nature and intensity of the course of fermentation processes is the quantitative assessment of changes in the carbohydrate complex (**Table 8.2**) and the content of total sugars (**Fig. 8.4**). In all studied samples, a decrease in the total content of carbohydrates is observed, indicating their active involvement in fermentation metabolism.

In legume crops, the decrease in carbohydrate content occurred at different rates. Thus, for chickpeas, the carbohydrate content after fermentation decreased by 10.3%, for red lentils by 9.7%. For green peas, the grains of which were characterized by the minimum initial value among legumes, it decreased by 15.4%.

In cereal and pseudocereal substrates, the decrease in carbohydrate content after fermentation was relatively stable and fluctuated within 1.73–1.95 g/100 g, which corresponds to the range of 7.4–9.2%.

In vegetable substrates, the decrease in carbohydrate content in absolute values was less pronounced and amounted to 0.55–1.04 g/100 g. However, the percentage comparison regarding the initial value indicated significant changes. In carrots, the level of carbohydrates became lower by 10.9%, in zucchini by 17.7%, and in tomatoes by 19.5%.

Table 8.2 Changes in the content of macronutrients in plant-based materials during fermentation

Plant-based material	Carbohydrates, g/100 g		Proteins, g/100 g		Fats, g/100 g	
	before fermentation	after fermentation	before fermentation	after fermentation	before fermentation	after fermentation
Chickpeas	19.66 ± 1.44	17.64 ± 1.58	6.56 ± 0.32	6.73 ± 0.58	1.93 ± 0.12	2.02 ± 0.04
Carrots	9.58 ± 0.69	8.54 ± 0.05	0.93 ± 0.02	1.00 ± 0.06	0.24 ± 0.03	0.25 ± 0.02
Oats	21.22 ± 2.64	19.27 ± 1.24	5.41 ± 0.06	5.51 ± 0.30	2.21 ± 0.05	2.25 ± 0.14
Tomatoes	3.89 ± 0.25	3.13 ± 0.03	0.88 ± 0.02	0.93 ± 0.02	0.20 ± 0.03	0.21 ± 0.01
Red lentils	19.23 ± 2.42	17.37 ± 1.46	8.26 ± 0.05	8.38 ± 0.34	0.36 ± 0.19	0.39 ± 0.01
Quinoa	20.54 ± 1.61	18.77 ± 1.41	4.51 ± 0.16	4.64 ± 0.43	1.96 ± 0.07	2.01 ± 0.05
Green peas	14.45 ± 1.07	12.23 ± 0.77	5.42 ± 0.03	5.63 ± 0.18	0.39 ± 0.05	0.49 ± 0.03
Zucchini	3.11 ± 0.17	2.56 ± 0.21	1.21 ± 0.08	1.28 ± 0.12	0.32 ± 0.02	0.35 ± 0.01
Barley	23.53 ± 2.08	21.80 ± 1.64	3.17 ± 0.24	3.28 ± 0.47	0.39 ± 0.04	0.42 ± 0.06

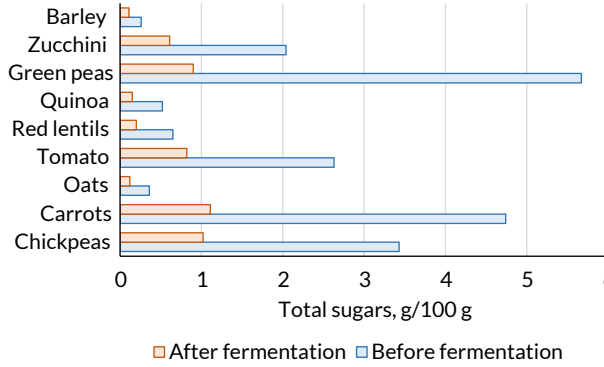

Fig. 8.4 Content of total sugars in plant substrates after fermentation, g/100 g

Analysis of the content of total sugars (**Fig. 8.4**) confirms their decrease after fermentation in all groups of plant substrates by 58–84%. It was most pronounced in green peas (84%), carrots (77%), quinoa (71%), chickpeas, zucchini (70%), and red lentils (69%). Minimal loss of sugars was observed during barley fermentation (58%). The results obtained indicate the direct involvement of mono- and disaccharides in metabolic processes during lactic acid fermentation, regardless of the species characteristics of the substrates. Along with this, it should be noted that in grain substrates (barley, oats) there is a significantly higher preservation of total sugars, which

indicates a more active involvement in metabolic processes of starch and other reserve polysaccharides.

Thus, the change in the carbohydrate complex during fermentation has several important technological values. On the one hand, it forms the sugar profile of future sauces and provides the necessary balanced taste properties, and on the other hand, it takes a direct part in the formation of their consistency and provides a viscous, homogeneous structure.

In order to establish the influence of fermentation on the nature of the restructuring of the protein complex of plant substrates during lactic acid fermentation, the content of total protein was determined. The results of experimental studies indicate an insignificant increase in the mass fraction of protein in all plant substrates. In substrates characterized by a higher natural protein content before fermentation, the increase in the indicator after fermentation was also more substantial. In particular, in chickpeas, the content of total protein increased by 0.17 g/100 g, quinoa by 0.13 g/100 g, red lentils by 0.12 g/100 g, barley by 0.11 g/100 g, and green peas by 0.21 g/100 g. At the same time, in vegetable substrates, the changes were minimal and did not exceed the level of statistical error.

Protein increase may be associated with the accumulation of microbial biomass, which increases the total content of nitrogenous substances. According to the chosen methodology, protein was determined by total nitrogen, the content of which characterizes the total content of nitrogenous substances, which takes into account both the content of partial proteolysis products and proteins of microbial origin involved in the fermentation process. In addition, during fermentation, structural rearrangement of proteins occurs. Under the action of proteases of lactic acid bacteria, partial hydrolysis of proteins occurs, and peptides and free amino acids are formed. This is accompanied by an increase in the bioavailability of amino acids and the formation of a specific umami taste component. At the same time, no decrease in the mass fraction of total protein is observed.

The results of the experimental study also established minimal changes in the mass fraction of fats in all plant substrates after fermentation. The increase in this indicator, recorded within 0.01–0.09 g/100 g, is within the statistical error and confirms its stability. Thus, it can be concluded that there is no lipolytic activity of lactic acid microorganisms during the fermentation of selected plant substrates. Preservation of lipid structural stability is an important condition for ensuring a homogeneous consistency of future sauces.

Therefore, based on the obtained results of experimental studies, it is concluded that a decrease in the proportion of carbohydrates is accompanied by an increase in the content of organic acids and a structural transformation of the protein-lipid

complex of plant substrates without the loss of their quantity. This increases the technological suitability of fermented plant substrates for the production of functional sauces with a predicted consistency, as well as taste and aroma characteristics.

8.5 Nutrient profile and energy value of sauces from fermented plant-based materials

Assessment of the nutrient profile (**Table 8.3**) is an important stage in substantiating the formulations of functional sauces, since proteins, fats, and carbohydrates determine not only the energy value of the product, but also form its consistency, taste profile, and emulsion stability. The obtained results demonstrate clear differences between the control sample and variants from fermented plant-based materials, confirming the influence of both the formulation composition and the preliminary biotechnological treatment.

Table 8.3 Content of essential macronutrients and chemical components in sauces from fermented plant-based materials

Indicator	Cf	FCCO	FGZB	FTLQ
Proteins, g/100 g	4.65 ± 0.08	3.39 ± 0.03	2.48 ± 0.03	2.79 ± 0.04
Fats, g/100 g	9.51 ± 0.10	9.20 ± 0.09	7.45 ± 0.03	7.60 ± 0.02
Carbohydrates, g/100 g	15.26 ± 0.05	11.89 ± 0.11	8.44 ± 0.37	11.67 ± 0.11
Sugars, g/100 g	2.50 ± 0.03	1.23 ± 0.13	0.99 ± 0.03	1.32 ± 0.03
Fiber, g	3.46 ± 0.03	3.58 ± 0.09	3.15 ± 0.10	5.03 ± 0.17
Titratable acids, %	0.36 ± 0.02	0.64 ± 0.03	0.67 ± 0.02	0.73 ± 0.04

In terms of total protein content, the control variant of the sauce exceeded all the experimental variants by an average of 1.4…1.9. The decrease in the mass fraction of protein in the samples of the experimental variants of the sauces is associated with the peculiarities of their formulation. Thus, the formulation of the control variant of the sauce includes 60% of boiled chickpeas, which is considered a high-protein plant ingredient. In contrast, the formulation of the experimental samples includes other fermented plant ingredients, the protein content of which is significantly lower. However, this difference does not affect the functional properties of the sauces, since they are not considered the main source of protein in the diet. It should also be noted that the complete or partial replacement of chickpeas in the experimental formulations has a positive effect on the digestive processes and facilitates the

digestibility of ready-made dishes. A smaller mass fraction of the protein fraction in the experimental samples of the sauces contributes to the formation of a more uniform consistency and reduces the risk of protein coagulation during their storage.

The fat content in all samples of sauces prepared according to the experimental formulations was lower compared to the control sample. Depending on the variant, the decrease amounted to 3.3...21.7% compared to the control variant. This had a positive effect on the functional properties of the sauces. In the experimental samples, the proportion of saturated fatty acids decreased, the lipid profile improved, and the energy value significantly decreased. It should also be noted that the improvement of the functional properties of sauces is facilitated not only by the quantitative composition of the fat fraction, but also by its qualitative composition. In the experimental sauce formulations, it is proposed to use various types of cold-pressed plant oils, which have undeniable nutritional advantages. Thus, olive oil is characterized by a high content of monounsaturated fatty acids, contains polyphenols and tocopherols, providing a high antioxidant potential. It is stable in cold emulsions and has high emulsifying compatibility with vegetable proteins. Pumpkin seed oil contains a lot of linoleic acid, tocopherols, and phytosterols. It has a natural nutty aroma, deep, rich taste, and color, and increases the viscosity of emulsions. Red basil oil contains volatile terpene compounds, enhances the aroma of sauces, and improves their color range.

Sauce samples prepared according to the control formulation exceeded all experimental samples both by carbohydrate content (by 1.3...1.8 times) and by total mono- and disaccharides content (by 1.9–2.5 times). Among the experimental samples, FCCO sauce samples had the maximum carbohydrate content, and FTLQ samples had the highest sugar content. FGZB sauce samples had the minimum content of total carbohydrates as well as mono- and disaccharides. The reduced mass fraction of carbohydrates, including readily available sugars, contributes to the formation of food products with a reduced glycemic load and limits the possibility of undesirable carbonyl stress.

A special advantage of sauces prepared according to the experimental FCCO and FTLQ formulations is the increased content of dietary fiber. In the FCCO sauce samples, their content exceeded the control variant by 1.1 times, and in the FTLQ sauce samples by 1.5 times. High level of fiber in these sauces is associated with the use of cereals, pseudocereals, and legumes, as well as the addition of inulin. This increases the functional properties of the sauces and makes it possible to consider the experimental samples of sauces as a source of prebiotic components. From a technological point of view, this ensures the formation of a thick, stable, homogeneous consistency of sauces.

The indicators of titrated acidity additionally confirm the functional orientation of the experimental samples. In the control variant, it was 0.36%, while in sauces from fermented plant-based materials, it increased to 0.64–0.73%. The highest level of acidity was recorded in FTLQ (0.73%), which is associated with the use of fermented tomatoes and lentils. The increase in acidity, combined with a reduced sugar content, forms a more pronounced, balanced flavor profile and contributes to the microbiological stability of the product.

Thus, the comparative analysis indicates that FCCO and FTLQ sauces based on fermented vegetable raw materials are characterized by a higher content of dietary fiber, a reduced level of fats, carbohydrates, and simple sugars. The combination of these indicators indicates the possibility of manufacturing products with improved functional properties.

The energy value of the developed sauces is presented in **Fig. 8.5**.

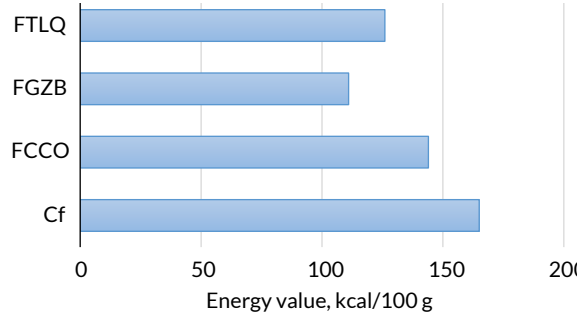

Fig. 8.5 Energy value of sauces formulated from fermented plant-based materials (kcal/100 g)

Assessment of the energy value of sauces (**Fig. 8.5**) reveals a consistent decrease in calorie content in all variants from fermented plant-based materials compared to the control sample. The maximum energy value was observed in the samples of sauces prepared according to the control formulations. The determined value of this indicator was 165 kcal/100 g of finished sauce. This is due to the high content of both boiled chickpeas and the liquid fat fraction – olive oil – in the formulation composition. According to the results of the studies (**Table 8.3**), the control samples of Cf sauces exceeded all samples of the experimental variants in terms of the content of proteins, fats, and carbohydrates. Therefore, the maximum energy value is quite expected.

The energy value of sauces prepared according to the experimental FCCO formulation was 144 kcal/100 g, i.e., it was 13% lower than the samples of the control

variant, but at the same time it exceeded all samples of other experimental variants. Such dynamics are due to the fact that in the FCCO formulation, chickpeas are partially replaced by lower-calorie products, e.g., carrots and oats. In addition, all the main plant ingredients are used in the fermented form, which results in a decrease in the content of easily accessible carbohydrates and, accordingly, a decrease in their contribution to the overall energy balance.

The minimum energy value was observed in the samples of sauces prepared according to the FGZB formulation. In this variant, the value was 111 kcal/100 g of the finished sauce, which is 33% lower than in the control variant. The calorie content of this sauce sample, as well as all other experimental variants, is provided by fermented ingredients of the formulations. In addition, the use of pumpkin oil in a smaller amount compared to the control also contributed to a decrease in energy value.

In the FTLQ variant, the energy value was 126 kcal/100 g. Despite the presence of lentils and quinoa as sources of protein and complex carbohydrates, the reduction in the content of simple sugars due to fermentation and a moderate proportion of fat provided a decrease in calorie content compared to the control by 24%. In addition, the low intrinsic energy value of fermented tomatoes, which are the basis of the formulation and contribute to a decrease in the overall energy load, should be taken into account.

Thus, the results obtained indicate that the introduction of fermented plant components into sauce formulations allows for a reduction in the energy value by 21–54 kcal/100 g compared to the control sample, while maintaining an increased content of protein and dietary fiber.

8.6 Organoleptic evaluation of sauces based on fermented plant-based materials

Organoleptic evaluation of sauces from fermented plant-based materials and the control sample was carried out using a nine-point scale, determining such indicators as appearance, color, consistency, aroma, and taste (**Table 8.4**). The results of the sensory evaluation indicate significant differences between the samples of sauces prepared according to the control formulation (Cf) and all experimental formulations.

The mean score of the sauce samples prepared according to the control formulation was 6.56 points. According to the tasters, the control samples were characterized by a mild neutral taste and aroma, which was reflected in the tasting score for these criteria – 6.49 and 6.42 points, respectively. The consistency of the sauces was paste-like, but not completely homogeneous, with a slight graininess and, according

to some experts, too thick for the sauce system. The color was uniform, light beige, but too neutral and insufficiently expressive. Thus, the scores for these criteria were also not maximum – 6.81 points for consistency and 6.5 points for color.

Table 8.4 Sensory indicators of sauces from fermented plant-based materials, scores

Sample	Appearance	Color	Consistency	Aroma	Taste	Mean score
Cf	7.03 ± 0.41	6.50 ± 0.04	6.81 ± 0.19	6.42 ± 0.06	6.49 ± 0.09	6.56 ± 0.41
FCCO	8.73 ± 0.19	8.52 ± 0.28	8.59 ± 0.25	8.38 ± 0.14	8.43 ± 0.13	8.48 ± 0.19
FGZB	8.44 ± 0.11	8.03 ± 0.14	8.32 ± 0.14	8.24 ± 0.15	8.30 ± 0.08	8.22 ± 0.11
FTLQ	8.90 ± 0.10	8.98 ± 0.05	8.82 ± 0.20	8.83 ± 0.12	8.90 ± 0.14	8.88 ± 0.10

The experts rated the sauce samples prepared according to the experimental FCCO formulation significantly higher. Their average score was 8.48 points. The maximum scores were given to the attractive appearance and homogeneous, moderately viscous, and stable consistency of the sauces (**Table 8.4**). The color of the sauces was natural with a light orange tint provided by the fermented carrot included in the formulation. The taste and aroma were balanced, sweet and sour, with light spicy notes.

The mean score of the sauce samples prepared according to the FGZB formulation was 8.22 points. That was the minimum score among all sauce samples prepared using the experimental formulations. However, it also exceeded the organoleptic evaluation of the control samples by 1.3 times (**Table 8.4**). The experts noted that the consistency of these sauces was homogeneous, delicate, and moderately thick. The color was light green, but less intense than other variants. Nevertheless, this did not have a significant impact on the appearance, and it remained quite attractive and was rated 8.44 points. The taste and aroma were inherent in the plant-based materials included in the formulation and characterized by freshness and a slightly sour taste.

The maximum scores were given to the sensory indicators of sauces prepared according to the experimental FTLQ formulation. The mean score of these samples was 8.88 points. The experts awarded the highest scores (**Table 8.4**) to the intense ruby color provided by the combination of fermented tomatoes and red lentils. This bright color resulted in an attractive appearance, which was rated at 8.9 points. High scores were also given to the pronounced taste and aroma profile of the sauces of the FTLQ variant, characterized by a successful combination of sweet-and-sour and spicy notes. Their consistency was thick, homogeneous, and without delamination.

Thus, the presented results of the organoleptic evaluation indicate that all samples prepared according to the experimental formulations exceed the samples prepared according to the control formulation by an average of 1.4–2.5 times for each

organoleptic indicator. The greatest impact of the plant-based material fermentation was observed in the taste and aroma characteristics of functional sauces.

A comparative assessment of organoleptic indicators of both the control and experimental variants showed that each sample made according to a unique formulation is characterized by a characteristic sensory identity. Thus, FCCO sauces had a balanced taste and aroma profile with light smoky-spicy notes. The taste and aroma profile of FGZB sauces was characterized by freshness, with a slight "green" plant-dominant tone and delicate sourness. The FTLQ sauces demonstrated the brightest taste and aroma profile with deep umami undertones and sweet-and-sour notes. The formation of these properties of sauces is directly related to the influence of lactic acid fermentation on the functional and sensory characteristics of the plant base of sauces.

Thus, changes in plant-based materials that occur as a result of the metabolic activity of lactic acid microflora during fermentation significantly change the sensory characteristics of sauces, contribute to the formation of a brighter and more expressive aroma, as well as a pleasant, balanced, and harmonious taste. An increase in the organic acid content, as well as the accumulation of other products of lactic acid fermentation, contributes to the formation of natural sweet-creamy, sour, spicy, and umami flavors, which improve the culinary appeal of finished sauces. At the same time, changes in the structural components of fermented plant-based materials form a stable and homogeneous consistency. The results obtained confirm the feasibility of using fermented plant-based materials as an effective tool for increasing the sensory and functional attractiveness of sauces for the food industry and the HoReCa segment.

8.7 Biochemical mechanisms of quality formation and practical aspects of implementing the technology of sauces from fermented plant-based materials

The scientific research is aimed at systematically substantiating the formulation and production technology of functional sauces, in which lactic acid fermentation of plant-based materials is used as a key biotechnological tool for forming the quality and stability of the product for the restaurant segment and the food industry. The research is based on the scientific hypothesis that the use of lactic acid fermentation of vegetable, legume, and grain substrates as a functional basis for sauces will simultaneously improve their organoleptic characteristics, increase nutritional and biological value, and form a technologically stable system without the use of synthetic additives.

The obtained results confirm the internal logic of this hypothesis.

During lactic acid fermentation, due to the increased metabolic activity of microorganisms, complex biochemical processes occur, which are characterized by an interconnected set of changes in the pH value, titrated acidity, redox potential, and carbohydrate complex. The degree of intensity of these changes characterizes the kinetics of the fermentation process. In view of this, it is proposed to control the fermentation processes in studies by the level of pH decrease and the increase in the content of titrated acids, with simultaneous comparison with the decrease in the content of total sugars and carbohydrates in general. This approach is consistent with the results of modern studies, in which these criterion indicators were selected to monitor the course of fermentation [33].

The indicator of active acidity pH has been selected as the main control criterion in our studies, as well as in scientific publications [7, 34]. During fermentation of plant substrates, in particular legumes, cereals, pseudocereals, and vegetable crops, the increase in pH is primarily caused by the lactic acid accumulation. When the level of active acidity pH $\leq$ 4.6 is reached, the growth of pathogenic microorganisms is significantly inhibited, they lose the ability to reproduce, and the products acquire higher microbiological resistance. Lactic acid bacteria are characterized by higher acid resistance; therefore, after reaching this acid barrier, they continue to participate in metabolic processes with the formation of lactic acid. However, as the content of the latter increases, their activity is gradually inhibited.

A comparative analysis of the obtained changes in titrated acidity with the data from other scientific studies [7] shows that the values are typical for fermented plant substrates. Thus, it is well known that the most pleasant and balanced taste is inherent in fermented vegetables with a content of titrated acids in the range of 0.6–0.8%. The results of our studies are fully consistent with such values and are within this range. According to the research [7], the intensity of lactic acid formation is determined by the preliminary preparation of plant substrates for fermentation, in particular, the degree of grinding and moistening if necessary. In view of this, all plant substrates used in our studies were ground to particles $\leq$ 5 mm in size, and dry ones were moistened to 68 $\pm$ 2%. Such technological techniques contributed to the intensification of fermentation processes and the achievement of the required acidity levels within 24 hours.

Simultaneously with changes in the acidity of the medium during lactic acid fermentation, changes in its redox potential occur. Due to increased metabolic activity, lactic acid bacteria consume certain types of reducing compounds, use the energy generated in this process, and form new substances. This leads to a gradual change in the redox state of the system. Reviews on the fermentation of plant substrates emphasize that the state of the redox system and the availability of oxygen determine

both the intensity of the fermentation process and the subsequent stability of the product during storage [35].

The study of the carbohydrate component of plant substrate is a logical continuation of the changes in acidity and redox potential. The decrease in the mass fraction of sugars after fermentation is fully consistent with the generally accepted scheme: mono- and disaccharides are the main substrates for lactic acid bacteria and determine the rate of acid accumulation. For legumes, the literature separately emphasizes a significant reduction in readily available sugars with a parallel improvement in their technological and nutritional characteristics. For cereals and pseudocereals, a scenario of partial starch hydrolysis is described, which may temporarily modify the proportion of low-molecular sugars and affect the rheology of the system [35].

It is these biochemical transformations in fermented plant-based materials that create the prerequisites for the formation of a qualitatively new profile of ready-made sauce systems.

Comparison of control and fermented sauces demonstrates the effect that is typical for plant-based fermented products: a decrease in carbohydrate content and an increase in dietary fiber content in formulations that combine legumes and cereal or pseudocereal fermented ingredients. Modern reviews confirm that lactic acid fermentation can increase nutritional value, technological quality, and functional properties by changing the availability of nutrients, reducing the proportion of antinutrients, and restructuring protein fractions [36].

The introduction of fermented legumes into the composition of sauce formulations significantly changes the protein complex. During fermentation in legumes, protein proteolysis occurs. The result of this process is the formation of peptides and amino acids. At the same time, the total amount of protein remains stable, but its structure becomes completely different. These changes improve the properties of the protein component, in particular, increase solubility, moisture retention capacity, improve emulsifying ability, change taste qualities, making them more saturated and harmonious [37]. These data are fully consistent with the higher scores of organoleptic evaluation of experimental sauce samples.

Crops such as oats, barley, and quinoa are characterized by a high content of starch, polysaccharides, and dietary fiber. As a result of fermentation, this carbohydrate complex is partially changed and restructured. The introduction of fermented grain and pseudo-grain raw materials into formulations increases the viscosity of sauces and provides a stable, homogeneous structure without delamination [38].

A completely predictable consequence of the obtained experimental research results, and primarily a decrease in the sugar content, is a decrease in the energy value of the experimental sauce samples compared to the control variant.

Thus, summarizing the obtained results, it can be concluded that lactic acid fermentation of plant-based materials can be an effective technological stage in the process of producing sauces with improved functional properties. The main advantages of these sauces are the regulated formation of organic acids and the creation of the necessary acidic environment, improvement of organoleptic indicators, and reduction of energy value.

Along with this, a detailed analysis of the results has identified a number of issues that remained unresolved and determined the prospects for further research. Thus, the issue of protein proteolysis and its consequences, as one of the main factors in the formation of the umami taste of functional sauces, requires further research. A more in-depth study of the influence of the oxygen environment on changes in the redox potential of plant-based materials during fermentation and the preservation of their stable natural color is also of great scientific interest. The microbiological stability of sauces and, accordingly, their food safety, both when manufactured in restaurant establishments and in food industry enterprises, requires experimental verification as well.

In general, the obtained results of experimental research demonstrate the possible high efficiency of the proposed technology for the production of sauces, both when implemented in the HoReCa segment and the food industry, taking into account all modern requirements of regulatory documentation and consumer preferences.

The proposed technology for the production of sauces for the HoReCa segment and food industry enterprises will be based on the process of preliminary lactic acid fermentation of plant-based materials, which, according to our research, is decisive in the formation of taste and quality characteristics of the finished product. The main difference of the developed technology is the biochemical mechanisms of formation of the necessary technological and organoleptic properties, in contrast to traditional technologies, which require stabilizers, flavor enhancers, aroma, dyes, and preservatives. In view of this, the finished product will meet the modern requirements of catering establishments and food enterprises to reduce the amount of synthetic food additives and comply with the Clean Label concept.

The technological scheme of the developed process is presented in **Fig. 8.6**.

Sauce production begins with raw material reception. It must meet all normative technological and sanitary requirements. Before the start of fermentation, plant-based materials at food industry enterprises are stored in refrigeration chambers or warehouses in compliance with the regime parameters listed in **Table 8.5**.

In the conditions of enterprises of the HoReCa segment, wet plant-based materials are stored in hermetically sealed containers with proper labeling in refrigerators according to the regime parameters given in **Table 8.6**. Dry raw materials are stored

in clean, dry warehouses in hermetically sealed production packaging or in hermetically sealed containers. Storage conditions and terms are shown in **Table 8.6**.

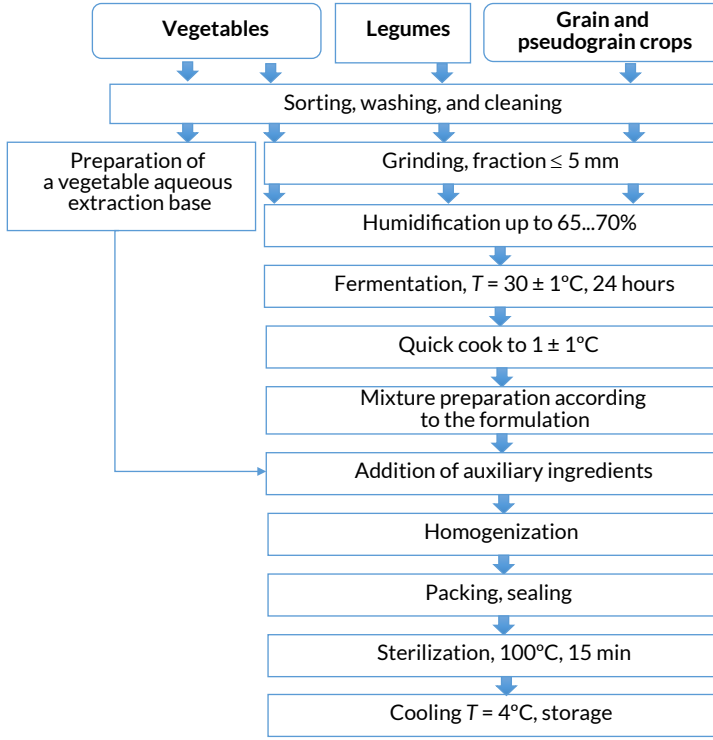

Fig. 8.6 Technological scheme for the production of functional sauces using fermented plant-based materials

The first stage of sauce production is the inspection and sorting of plant-based materials. At the same time, they remove raw materials that do not meet technological requirements, i.e. with signs of microbiological and physiological deterioration, mechanically injured, etc. In addition, various impurities are removed. Then the vegetables are washed. Water for washing raw materials must meet all sanitary requirements for drinking water.

Next, all plant-based materials are crushed to a fraction size of ≤ 5 mm. Grinding is carried out in the conditions of food industry enterprises using hammer crushers or roller or disk mills, in the conditions of catering establishments, on universal

kitchen grinders, mills, blenders, and cutters. The purpose of grinding is to increase the availability of nutrients of plant substrates for lactic acid microflora.

Table 8.5 Storage conditions and terms for plant-based materials in the conditions of food industry enterprises

Plant-based material	Storage conditions		Shelf life
	temperature, °C	relative air humidity, %	
Carrots	0…2	90…95	2…4 months
Tomatoes	8…12	90…95	5…10 days
Zucchini	4…8	90…95	7…10 days
Green peas	0…2	95…98	2…5 days
Chickpeas	5…15	≤ 70	12…24 months
Red lentils	5…15	≤ 70	12…18 months
Quinoa	5…15	≤ 65	12…18 months
Spelt wheat	5…15	≤ 70	12…24 months
Oat	5…15	≤ 70	8…12 months
Barley	5…15	≤ 70	12…18 months

Table 8.6 Storage conditions and terms for plant-based materials in the conditions of HoReCa establishments

Plant-based material	Temperature of storage, °C	Shelf life
Carrots	2…4	5…10 days
Tomatoes	6…10	3…7 days
Zucchini	4…6	3…5 days
Green peas	2…4	1…2 days
Chickpeas	10…18	6…12 months
Red lentils	10…18	6…12 months
Quinoa	10…18	6…12 months
Spelt wheat	10…18	6…12 months
Oat	10…18	4…6 months
Barley	10…18	6…12 months

After grinding, the dry raw material was moistened with water at a temperature of 30–40°C to a moisture content of 65–70%. The purpose of this technological operation was to activate plant enzymes and create more favorable conditions for the intensive development of lactic acid microflora.

The prepared plant substrates were sent for fermentation. Fermentation was carried out with pure cultures of lactic acid bacteria of the species Lactiplantibacillus plantarum, which were added at a concentration of 10^6–10^7 CFU/g. The temperature during fermentation in thermal chambers or thermostats was maintained within 30 ± 1°C. Such temperature conditions are optimal for the development of this type of lactic acid bacteria and maximally reduce the risk of the development of foreign microflora. The process was stopped after 24 hours when the pH of the substrates decreased to ≤ 4.6 by rapid cooling to a temperature of 1 ± 1°C in intensive cooling chambers – in production conditions, or in shockers – in food establishments. A sharp drop in temperature to the limit of 0...1°C causes the inactivation of vegetative forms of microorganisms and stops the process of lactic acid fermentation. It should also be noted that the absence of heat treatment and the use of particularly low positive temperatures for the inactivation of microflora ensures significantly higher preservation of natural biologically active substances of plant-based materials and increases their functional properties.

When fermentation is stopped, plant ingredients in sealed containers should be stored at 1 ± 1°C in refrigeration chambers or refrigerated wall cabinets until they are used to prepare sauces, but no more than 7 days.

The formulation of functional sauces from fermented plant-based materials includes a liquid base, i.e., vegetable broth, which is prepared in advance. Carrots, onions, and celery root are used for their preparation. Vegetables are inspected, washed, cleaned, chopped into pieces 10...15 mm in size, and poured with drinking water in the ratio: 3 parts of water are added to 1 part of vegetables. Next, the mixture is brought to a boil and cooked at a slow boil for 30 minutes. After cooking, the liquid part is separated from the cooked vegetables and filtered. The obtained vegetable broth is cooled to a temperature of 18–20°C in the case of immediate use for preparing sauces, or to 1 ± 1°C for storage in refrigeration conditions for no more than 24 hours.

Sauces are prepared by mixing all the ingredients provided by the developed formulations in the homogenizer following a specific sequence: first, vegetable broth is introduced into the homogenizer, then fermented plant-based materials are added. The mixture is homogenized at a shaft rotation speed of at least 3000 rpm for 3–5 minutes until a stable, homogeneous consistency is formed. Inulin is introduced (for the FTLQ variant) and homogenized for 2 minutes. The fat phase is introduced and homogenize to form a stable homogeneous emulsion. The acidity is adjusted by adding lemon juice or the specified types of vinegar. Then, it is necessary to homogenize for 2 minutes. Salt, spices, garlic, and herbs are added. Then, homogenize to form a uniform stable consistency. The preparation of the sauce is finished

by checking the organoleptic properties. The finished product is packaged in a prepared sterile container and sealed.

When preparing sauces in HoReCa establishments, the finished product is cooled to 4 ± 1°C and stored in a refrigerator at 1 ± 1°C for up to 5 days.

When preparing sauces in the conditions of food industry enterprises, after sealing, they are sterilized at 100°C for 15 minutes. During sterilization, pathogenic microflora is destroyed, which helps to extend the shelf life of ready-made sauces without the use of a cold chain. Sterilized sauces have a lower content of biologically active substances compared to fresh ones that have not undergone heat treatment, but retain a stable consistency, high functional properties, and excellent organoleptic indicators.

Therefore, implementing the developed technology will ensure the production of premium-quality sauces for HoReCa establishments, as well as sauces with an extended shelf life and high functional properties for mass industrial production and sale in the retail network.

The technological and functional advantages of the developed sauce production technology are confirmed by economic calculations (**Table 8.7**).

Table 8.7 The main economic indicators of the developed sauce technology from fermented plant-based materials

Indicators	Implementation	Cf	FCCO	FGZB	FTLQ
Cost price, UAH/kg	HoReCa	189	152	163	223
	industrial production	246	224	235	301
Sales price, UAH/kg	HoReCa	833	1000	1000	1333
	industrial production	500	500	500	650
Profit, UAH/kg	HoReCa	644	848	837	1110
	industrial production	254	276	265	349
Level of profitability, %	HoReCa	341	558	515	498
	industrial production	103	123	112	116

The results of the calculations (**Table 8.7**) show that the cost of the sauce, which is made according to the control formulation based on boiled chickpeas (Cf) in the HoReCa segment, was UAH 189 per kg and was higher than the cost of the experimental variants. This is due to the use of a larger amount of fairly valuable chickpeas and the higher energy intensity of the process of its preparation. Partial or complete replacement of chickpeas with cheaper local raw materials for Ukraine, as well as the replacement of cooking with fermentation, helps to reduce the cost of experimental

samples. The maximum cost of FTLQ sauce is associated with the use of raw materials (quinoa, red lentil, red basil oil, inulin) of a higher price segment.

A comparative analysis of the cost price reveals a higher level of this indicator in industrially produced sauces compared to similar sauces in the HoReCa segment by 31–46%, depending on their type. Such an excess is explained by additional costs for heat treatment, packaging, labeling, quality control, logistics, wages, etc. At the same time, the ratio between the experimental samples fully corresponds to HoReCa sauces.

Better organoleptic properties of the sauce samples, prepared according to the experimental FCCO and FGZB formulations, made it possible to set higher sales prices in the menu of establishments, which, in turn, ensured a higher level of profit (**Table 8.7**). Cf, FCCO, FGZB industrially produced sauces had a similar selling price. However, the lower cost of the experimental FCCO and FGZB samples made it possible to obtain higher profits compared to Cf sauce. As for FTLQ sauce, it is positioned as a premium quality product in terms of functional and sensory properties. This made it possible to set a higher selling price and, therefore, to obtain a higher profit compared to all other options.

The evaluation of the level of profitability (**Table 8.7**) reflects the cost-effectiveness and characterizes the economic advantages of each sauce sample. All sauce samples, which were made according to experimental formulation, had a higher level of profitability compared to the control. The maximum level of profitability was determined in FCCO sauce, which provides it with better resistance to fluctuations in the purchase prices of raw materials and production costs.

Thus, the obtained results confirm the expediency of the production of sauces according to the developed technology based on the use of fermented plant-based materials in the conditions of the food industry and the HoReCa segment, both from the standpoint of technological and functional advantages, as well as economic effectiveness.

An important direction of further research can be the assessment of the bioavailability of mineral substances, antioxidant activity and prebiotic potential of products during the fermentation of plant-based materials, the study of microbiological stability and shelf life during the storage of ready-made sauces, as well as the approbation of the technology in the conditions of scaling and practical implementation in the conditions of craft productions of the food industry and institutions of the HoReCa segment.

The results obtained in the research process constitute an essential scientific and practical basis for the creation of innovative products, in particular in the field of sauce production, and provide opportunities for expanding their assortment.

8.8 Conclusions

As a result of the simulation of three formulations of fermented sauces using chickpeas, carrots, oats, lentils, quinoa, tomatoes, zucchini, barley, and green peas, functional products with a balanced composition were created. The choice of raw materials is based on both nutritional value and the ability to undergo enzymatic transformation.

An assessment of physicochemical changes in plant-based materials before and after fermentation was carried out, with an emphasis on determining pH, redox potential, titrated acidity, protein, fat, total carbohydrates, and sugars. A significant decrease in pH was established, which indicates an active accumulation of organic acids. At the same time, a shift of the redox potential towards a reducing environment was recorded, which is typical for anaerobic fermentation conditions, especially in samples with a high sugar content. A substantial decrease in the total content of carbohydrates, in particular sugars, was observed as a result of their utilization by microorganisms. The obtained changes indicate an improvement in the functional properties of raw materials after fermentation.

The energy value of ready-made sauces based on selected formulations was determined. It was established that samples from fermented plant-based materials have a lower energy value compared to the control, which is associated with the biochemical degradation of part of the sugars and the partial splitting of organic compounds under the action of the enzymatic activity of microorganisms.

The organoleptic evaluation confirmed the clear formation of new taste profiles in sauces from fermented plant-based materials: a balanced taste with smoky-spicy notes (FCCO), a fresh green profile with a light texture (FGZB), and a pronounced umami taste with sweet-sour tones (FTLQ). All variants showed a highly stable and homogeneous consistency.

Based on the results of experimental research, a technology for the production of functional sauces from fermented plant-based materials for the food industry and HoReCa was developed.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper, as well as the published research results, including the financial aspects of conducting the research, obtaining and using its results, as well as any non-financial personal relationships.

Financing

The study was performed without financial support.

Data availability

The data that support the findings of this study will be made available by the authors on reasonable request.

Use of artificial intelligence statement

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

Authors' contributions

Marina Serdyuk: Supervision, Conceptualization, Methodology, Writing – original draft, Investigation, Project administration.

Valentyna Bandura: Conceptualization, Methodology, Writing – original draft, Investigation.

Tetiana Kolisnychenko: Methodology, Writing – original draft, Writing – review & editing, Investigation, Visualization, Validation.

Karina Palamarek: Writing – original draft, Writing – review & editing, Visualization, Formal analysis, Validation.

Olha Romanovska: Writing – original draft, Visualization, Formal analysis, Validation.

Tetiana Brykova: Writing – review & editing, Investigation, Visualization.

Tetiana Marusiak: Investigation, Formal analysis, Validation.

Anastasia Parashchuk: Resources, Formal analysis, Visualization.

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CHAPTER 9

Impact of long-term storage on the quality of frozen pickled sweet peppers

Nadiia Zahorko
Igor Dudarev
Valentyna Tkachuk

Abstract

Vegetables are produced seasonally; therefore, freezing is one of the main methods of preserving them while maintaining their quality. Both fresh vegetables and semi-finished or ready-to-eat vegetable products are commonly frozen. Such products are popular among consumers, as they provide rapid preparation and consumption. Vegetables, particularly sweet peppers, are often pickled in marinades with various spices to enhance sensory properties, preserve nutrients, extend shelf life, and expand the range of available products. A promising approach for storing pickled sweet peppers is freezing. In this study, sweet peppers were pickled in a marinade containing the following ingredients: water, sunflower oil, sugar, natural honey, salt, citric acid, and spices (dried dill and parsley, bay leaves, fresh garlic, and allspice). After washing with water, cleaning, and cutting, sweet pepper slices were pickled in the marinade for 12 h at room temperature and subsequently frozen at –20°C. Experimental results showed that during 270 days of frozen storage of pickled sweet peppers, losses of ascorbic acid ranged from 12.0 to 19.8%, carotenoids from 5.8 to 15.1%, dry matter from 0.7 to 4.9%, total sugars from 0.6 to 6.1%. At the same time, the contents of water-soluble pectin, anthocyanins, catechins, and total flavonoids increased during storage. Freezing also had a positive effect on the microbiological safety of the product, as the number of microorganisms in the frozen product during storage was lower than that in fresh sweet peppers. The sensory quality of the frozen product, including appearance, color, aroma, taste, and consistency, was highly rated by expert evaluators. To bring the product quickly to a ready-to-eat state, microwave thawing is recommended. Pickled sweet peppers thawed using this technique exhibited high sensory quality. However, prolonged storage of the product at room temperature after thawing is not recommended, as it leads to

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a decrease in ascorbic acid content and an increase in polyphenol oxidase activity and microbial growth. Although the product remained safe for consumption after 24 h of storage at 20°C, the number of microorganisms did not exceed the permissible limits for quick-frozen vegetables (the maximum bacterial and mold counts in the thawed pickled sweet peppers were 48,400 CFU/mm^2 and 19.0 CFU/mm^2, respectively).

Keywords

Sweet pepper, marinade, freezing, thawing, pickled vegetable, frozen vegetable, thawed vegetable, microwave-thawed vegetable, microflora, oxidative enzymes.

9.1 Introduction

Freezing is a widely used method for preserving vegetables [1]. This preservation method extends the shelf life of vegetables and helps maintain their quality. The use of freezing storage (below –20°C) increases the seasonal availability and distribution range of vegetables beyond the region where they are grown [2]. Physicochemical parameters, sensory attributes, and the texture of frozen vegetables are strongly affected by the freezing process. The most commonly used methods for freezing vegetables include air-blast, fluidized bed, immersion, and cryogenic freezing [3]. Conventional freezing method is characterized by relatively low process efficiently [4]. During freezing and frozen storage, vegetable cells are damaged, which leads to the degradation of released antioxidant compounds through chemical and enzymatic oxidation reactions [5]. Among the methods of pre-processing vegetables before freezing, blanching is commonly used, as it preserves the natural taste and color by inactivating enzymes. However, it also leads to the reduction in the content of vitamin C and other heat-labile compounds due to leaching into the blanching water [6]. To shorten the freezing process and prolong the shelf life of vegetables, osmotic dehydration is used as a pretreatment. During this process, vegetables are soaked in an aqueous solution containing sugar or salt [7]. Air-drying and osmodehydrofreezing are also used to remove a portion of water from vegetables prior to freezing [8]. Alternatively, so-called "chemical" blanching (using chemicals) and microwave-assisted blanching can be effectively applied prior to freezing to retain nutritional compounds, such as vitamin C [9]. Magnetic-field-assisted freezing helps enhance the freezing rate and improve the quality of frozen foods in terms of sensory properties compared to other freezing methods [10]. Freezing time can be shortened by 15–26%, and the quality of frozen products can be improved when combined electric- and magnetic-field-assisted freezing is applied to the freezing and thawing of vegetables [11].

Sweet pepper (*Capsicum annum* L.) is one of the most important fruit crops, containing biologically active compounds such as antioxidants, ascorbic acid, carotenoids, and vitamin E [12]. The shelf life of fresh sweet pepper fruits varies from 4 to 21 days and is limited by decay and microbial growth. It is also influenced by fruit cultivar, physiological maturity, and storage conditions [13]. Freezing is an important method for extending the shelf life of sweet peppers, which helps preserve the nutritional and sensory qualities of the fruits [14]. Storage temperature and humidity have a significant influence on the quality of sweet pepper fruits, including water loss rate and texture [15]. Pepper fruits are highly susceptible to chilling injury at temperatures below 7–10°C; therefore, different methods are used to alleviate chilling injury, including modified atmosphere, hot water, and chemical treatments [16]. The combined treatment (hot water at 45°C for 15 min + methyl salicylate at 0.05 mmol/L) is also used, as it helps reduce chilling injury and the loss of ascorbic acid and total phenolics [17].

Conventional pickling is a widely used method for enhancing the texture, color, and flavor of foods [18]. For sweet pepper pickling, an acidic marinade is used, consisting of vinegar, salt, sugar, bay leaves, allspice berries, and black pepper [19]. Advanced vegetable pickling techniques promote probiotic growth, enhancing the activity and stability of bioactive compounds, and improving product quality and health benefits [20]. To improve the quality and nutritional value of pickled peppers, they are fermented with dairy ingredients or by-products of the cheese-making process [21]. One novel technique for processing vegetables is pickling them using lactic acid bacteria (*Levilactobacillus brevis*, *Limosilactobacillus fermentum*, and *Lactiplantibacillus plantarum*), which results in increased vitamin C and total polyphenols [22].

For consumption or culinary use, frozen vegetables are thawed. If this process is performed incorrectly, it can significantly reduce the quality and nutritional value of vegetables. Conventional vegetable thawing techniques include ambient-temperature thawing and water thawing. In addition, a novel method has been developed, namely combined microwave- and infrared-assisted thawing, which helps shorten the thawing time by 65–75% and reduces thawing losses by 10–40% [23]. To reduce drip loss and maintain the sensory quality of foods, high-pressure, ultrasound-assisted, ohmic, radiofrequency, and vacuum-assisted thawing techniques have also been developed [24]. Defrosting at room temperature is a very slow process, and if the temperature is above 5°C, it creates favorable conditions for the rapid growth of harmful microorganisms [25]. Therefore, rapid thawing techniques are preferred over slower ones to obtain a safe product.

The intensive lifestyle of most urban populations has increased the popularity of frozen semi-finished products, which permit quick meal preparation. Consequently,

the food industry continues to expand the range of such products. The production of plant-based semi-finished products with maximal preservation of nutrients and sensory properties is particularly promising. In this context, the development of technology for producing frozen pickled sweet peppers that ensures rapid meal preparation and extended product shelf life is of considerable interest.

The main problem is that freezing, low-temperature storage, and subsequent thawing of vegetables can cause damage to cellular structures, leading to moisture loss, texture deterioration, and a reduction in the content of biologically active compounds. Therefore, the technology for producing frozen pickled vegetables, particularly sweet peppers, requires further investigation into the effects of freezing, storage conditions, and thawing methods on product quality and safety.

The aim of the study is to investigate the nutritional value, sensory attributes, and safety properties of frozen pickled sweet peppers during low-temperature storage and after thawing using different techniques.

9.2 Materials and methods

For the study, ripe fresh red sweet peppers without visible damage were used. The peppers were washed with water, the seeds and stalks were removed, and the pulp was cut into slices along the fruit. The peppers were blanched in hot water for 1–2 min.

The marinade contained (per 1 kg of sweet pepper slices): water (400 g), sunflower oil (80 g), sugar (50 g), natural honey (40 g), salt (15 g), and spices (dried dill and parsley (2 g), bay leaves (1 g), fresh garlic (4 g), and allspice (1 g). In the marinade, milder citric acid (4 g), which has no pungent odor, was used instead of traditional acetic acid. To prepare the marinade, water was boiled and sugar, salt, and spices were added and heated until the sugar and salt were dissolved. Citric acid and honey were then added, the mixture was boiled for 2–3 min, and the pepper slices were poured with the hot marinade. The pH of the marinade was 3.9–4.0. The peppers were pickled in the marinade for 12 h at room temperature (20°C). After pickling, the pepper slices with marinade were placed in plastic containers, and frozen at −20°C, at which temperature the product was stored.

Samples of pickled sweet pepper were analyzed before freezing and after freezing during storage (10, 90, 180, and 270 days) at a temperature of −20°C. For the analysis of sensory and physicochemical properties, as well as bioactive compounds, frozen pickled sweet pepper samples were thawed in air at 20°C.

The sensory parameters of pickled sweet pepper, dry matter content, sugar content, titratable acidity, ascorbic acid content, carotenoid content, flavonoid content,

pectin content, and microbiological parameters were determined. In addition, the sensory parameters of pickled sweet pepper were evaluated after thawing using different techniques (in air at room temperature, in water, and in a microwave oven).

A panel of seven trained experts evaluated the sensory attributes (appearance, color, aroma, consistency, and taste) of pickled sweet pepper samples using a 5-point scale according to method described in [26].

The dry matter content of pickled sweet peppers was determined according to method [27]. About 5 g of homogenized pickled peppers or frozen pickled pepper samples were weighed and evaporated to dryness in a SESH-3MU drying oven at 105°C for 5 h. The samples were then cooled in a desiccator and weighed. The dry matter content was expressed as a percentage of the initial weight of the sample. The dry matter content of the marinade was determined in a similar manner; however, the samples were dried until a constant weight was achieved.

The total sugar content of pickled and frozen pickled sweet peppers was determined according to the modified phenol-sulfuric acid method [28]. Briefly, 1 g of finely chopped pepper pulp was homogenized and extracted with 10 mL of 80% ethanol at 80°C for 30 min. The extract was filtered, and 1 mL of the filtrate was mixed with 1 mL of 5% phenol solution, followed by the rapid addition of 5 mL of concentrated H_2SO_4. The mixture was incubated at room temperature for 20 min, and the absorbance was measured at 490 nm using a ULAB 108UV spectrophotometer. Total sugar concentration was calculated from a calibration curve constructed with a glucose standard.

Titratable acidity, expressed as a percentage of citric acid, of pickled and frozen pickled sweet peppers was determined according to the method [29]. For the determination of titratable acidity, the samples were homogenized with distilled water and titrated with 0.1 N NaOH using phenolphthalein as an indicator. The titration endpoint was defined as the appearance of a persistent pink color. The results were expressed as a percentage of citric acid equivalents.

Ascorbic acid was extracted from the samples using meta-phosphoric acid with the addition of a small amount of acid-washed quartz sand. The resulting supernatant was then titrated with standard 2,6-dichlorophenolindophenol [27].

Carotenoids in pickled sweet peppers was determined according to [30]. Dried samples were extracted with acetone using a homogenizer until the filtrates were completely decolorized. The extract was washed with diethyl ether and separated with 10% NaCl. The organic phase was dried over anhydrous Na_2SO_4 and saponified with 20% KOH in methanol for 1 h at 20°C. The pigments were re-extracted with diethyl ether, evaporated, and dissolved in acetone (10 mL). Aliquots (1 mL) were centrifuged at 12,000 rpm and stored at −20°C until analysis. The carotenoid profile

of the extract was quantified using β-apo-8′-carotenal as an internal standard. The carotenoid profile was determined by HPLC using a Shimadzu LC-20AT Prominence system. The mobile phases used were 81:15:4 methanol/methyl tert-butyl ether (MTBE)/H_2O (solution A) and 91:9 MTBE/methanol (solution B). Gradient elution was performed from 100% A to 50% A and 50% B in 45 min, followed by 100% B in the next 10 min and 100% A in the next 5 min at a flow rate of 0.8 mL/min. Carotenoids were monitored at 450 nm.

Water-soluble pectin content in pickled sweet peppers was determined according to [31], with minor modification. Briefly, 25 g minced peppers were homogenized in 95% ethanol for 3 min, filtered, and the filtrate was washed twice with 75% ethanol. The solid residue was treated with 15 mL EDTA-Na_2, and the pH was adjusted to 11.5 with 0.1 M NaOH and incubated for 30 min. The solution was then adjusted to pH 5.5 with 2 N acetic acid, 15 mL of water was added, and the mixture was incubated at 50°C for 30 min. After filtration, the filtrate was made up to 50 mL and used as the water-soluble pectin extract. For analysis, 1 mL of extract was mixed with 6 mL H_2SO_4, boiled for 20 min, cooled, and then 0.2 mL of 0.15% carbazole was added, followed by incubation at 20°C for 2 h. Absorbance was measured at 520 nm using a ULAB 108UV spectrophotometer, and pectin content was calculated from a calibration curve prepared with a standard pectin solution.

Anthocyanin content in pickled sweet pepper was determined using the pH differential method [32]. The pepper pulp was ultrasonically extracted with methanol containing 1% HCl, and the supernatant was collected. Absorbance was measured at 530 nm and 700 nm using a ULAB 108UV spectrophotometer. Anthocyanin concentration was calculated according to the equation described in [32].

Catechin content in pickled sweet pepper was determined by HPLC [33] using a Shimadzu LC-20AT Prominence system equipped with a C18 column (250 × 4.6 mm, 5 µm) and UV detection at 280 nm. Samples were extracted with a methanol-water solution (70:30, v/v) containing 0.1% formic acid and filtered through a 0.22 µm membrane. Separation was performed using a gradient of water with 0.1% formic acid and acetonitrile at a flow rate of 1.0 ml/min. Catechin was identified by comparison with a catechin standard and quantified using a calibration curve.

The total flavonoid content in pickled sweet pepper samples was determined using a spectrophotometric method [34] based on the reaction with aluminum chloride ($AlCl_3$). A calibration curve was constructed using quercetin-3-rhamnoglucoside as a standard. About 0.5 mL of diluted extract was mixed with 0.4 mL of 25% ethanol and 0.5 mL of 10% sodium acetate. The mixture was kept in the dark at 20°C for 30 min, and the absorbance was measured at 430 nm using a ULAB 108UV spectrophotometer.

Microbiological analyses were performed according to the method [35]. About 25 g of pickled sweet peppers were homogenized in 225 mL of peptone water. Decimal dilutions were prepared and inoculated onto the media to enumerate aerobic mesophilic bacteria, yeasts, and molds. Bacteria were cultured on plate count agar, while yeasts and molds were cultured on yeast extract glucose chloramphenicol agar.

Polyphenol oxidase activity in pickled sweet pepper samples was determined spectrophotometrically according to a modified method described in [36]. Briefly, 1 g of pepper pulp was homogenized in 10 mL of 0.1 M sodium phosphate buffer (pH 7.0) containing 2 mM ascorbic acid. The homogenate was centrifuged at medium speed for 15 min at 4°C, and the supernatant was collected as the enzyme extract. The reaction mixture consisted of 2.8 mL of 0.1 M phosphate buffer containing 20 mM catechol as the substrate and 0.2 mL of enzyme extract. The increase in absorbance at 420 nm due to quinone formation during substrate oxidation was monitored using a ULAB 108UV spectrophotometer. Polyphenol oxidase activity was calculated from the rate of absorbance increase and expressed as μmol of substrate oxidized per minute per gram of fresh weight.

All analyses were carried out in triplicate. Statistical analysis was performed using Mathcad software to calculate mean values and standard deviations.

9.3 Frozen pickled sweet peppers

9.3.1 Sensory properties of frozen pickled sweet peppers

Table 9.1 presents the results of the sensory evaluation of pickled sweet peppers before freezing and during low-temperature storage after freezing.

Table 9.1 Sensory evaluation of pickled sweet peppers before freezing and during low-temperature storage after freezing (points)

Storage (days)	Sensory evaluation (mean)					
	Appearance	Color	Aroma	Taste	Consistency	Average score
0	4.9	5.0	5.0	5.0	5.0	4.98
10	4.8	5.0	5.0	4.6	4.3	4.74
90	4.8	5.0	5.0	4.7	4.4	4.78
180	4.9	5.0	5.0	4.9	4.7	4.90
270	4.9	5.0	5.0	4.9	4.8	4.92

Storage conditions did not affect the color or aroma of the peppers. The color of the peppers remained bright red throughout storage, and the aroma was typical of pickled peppers, with notes of the spices used. The appearance was rated 4.8 points on days 10 and 90 of storage, slightly lower than at the beginning and end of the storage period. According to expert assessments, the taste of the peppers deteriorated at the beginning of storage but improved by the end of the storage period. A similar trend was observed for changes in pepper consistency. During storage, the pulp of the pepper slices softened due to low temperatures; yet they retained their shape and elasticity. The taste of the product was typical of pickled peppers. The average sensory score was highest before storage (4.98), lowest on day 10 of storage (4.74), and increased to 4.92 points by day 270.

9.3.2 Physicochemical properties of frozen pickled sweet peppers

The dry matter content of pickled sweet peppers before storage was 5.92% (**Table 9.2**). During storage, it decreased to 95.1% of the initial value (5.63%) by day 270. Similarly, the dry matter content of the marinade decreased from 3.50% before low-temperature storage to 3.38% by day 180 and 3.40% by day 270. For comparison, the dry matter content of fresh sweet peppers ranges from 8.4 to 13.3% [27]. Thus, the dry matter content decreases during the pre-treatment and pickling stages.

During 270 days of storage, the total sugar content of frozen pickled peppers decreased to 93.9% of its initial value, from 5.43% to 5.10% (**Table 9.2**). Fresh sweet pepper fruits have a total sugar content ranging from 2.40 to 5.86% [29]. The increased sugar content in pickled sweet peppers is due to the addition of sugar to the marinade.

Table 9.2 Physicochemical parameters of pickled sweet peppers before freezing and during low-temperature storage after freezing

Storage (days)	Dry matter content (%)			Sugar content (%)		Titratable acidity (%)
	in pepper fruits	in marinade	preserved in peppers (%)	total	preserved in peppers (%)	
0	5.92 ± 0.08	3.50 ± 0.22	–	5.43 ± 0.17	–	0.31 ± 0.02
10	5.88 ± 0.34	3.47 ± 0.08	99.3	5.40 ± 0.28	99.4	0.30 ± 0.01
180	5.82 ± 0.11	3.38 ± 0.15	98.3	5.21 ± 0.23	95.9	0.30 ± 0.01
270	5.63 ± 0.15	3.40 ± 0.14	95.1	5.10 ± 0.24	93.9	0.32 ± 0.02

The total acidity of the frozen product did not change significantly during storage, remaining between 0.30% and 0.32% (**Table 9.2**). The titratable acidity of fresh sweet pepper ranges from 0.16 to 0.23% [29]. The acidity of pickled pepper was higher due to the addition of citric acid to the marinade.

9.3.3 Bioactive compounds of frozen pickled sweet peppers

The content of bioactive compounds in pickled sweet peppers before freezing and during low temperature storage after freezing is presented in **Table 9.3**. The ascorbic acid content of pickled sweet peppers was 226.7 mg/100 g. During storage, it decreased to 181.8 mg/100 g by day 270. Thus, at the end of long-term storage (day 270), the ascorbic acid content was 80.2% of the initial value, indicating that slow degradation occurs even at low temperatures. The vitamin C content in fresh sweet peppers, depending on the variety and growing conditions, ranges from 115.5 to 239.8 mg/100 g [27]. Thus, frozen pickled pepper maintain a relatively high level of ascorbic acid. A decrease in its content is also observed during the pre-treatment stage, particularly during blanching, when part of the water-soluble vitamin C passes into the water.

Table 9.3 Content of bioactive compounds in pickled sweet peppers before freezing and during low-temperature storage after freezing (mg/100 g)

Bioactive compounds	Storage (days)				
	0	10	90	180	270
Ascorbic acid	226.7 ± 0.6	199.6 ± 0.4	192.4 ± 0.2	187.3 ± 0.1	181.8 ± 0.6
Carotenoids	22.5 ± 0.3	21.2 ± 0.5	20.1 ± 0.2	19.4 ± 0.8	19.1 ± 0.4
Water-soluble pectin	417.4 ± 0.6	635.5 ± 0.3	796.4 ± 0.4	607.8 ± 0.3	513.1 ± 0.4
Anthocyanin	0.87 ± 0.07	1.04 ± 0.09	2.02 ± 0.07	1.56 ± 0.11	1.48 ± 0.22
Catechin	63.2 ± 0.3	66.2 ± 0.1	129.6 ± 0.5	131.5 ± 0.2	133.1 ± 0.2
Total flavonoids	144.6 ± 0.3	156.6 ± 0.3	315.7 ± 0.4	312.7 ± 0.1	347.7 ± 0.4

In pickled sweet peppers, the carotenoid content was 22.5 mg/100 g and decreased during low-temperature storage. After 270 days of storage, the carotenoid content decreased by 15.1%, reaching 19.1 mg/100 g (**Table 9.3**). The carotenoid content in fresh sweet peppers depends on the variety and stage of maturity, ranging widely from 14.1 mg/100 g (*Red Lamuyo* peppers) to 323.1 mg/100 g (organic peppers) [30].

The content of water-soluble pectin in frozen pickled peppers increased during storage for up to 90 days, from 417.4 mg/100 g (unfrozen pickled peppers) to 796.4 mg/100 g (frozen pickled peppers), and then decreased to 513 mg/100 g after 270 days of storage (**Table 9.3**). However, on day 270 of storage, the water-soluble pectin content in the peppers was still 22.9% higher than its content in unfrozen pickled peppers. This may be due to cell structure destruction and partial conversion of protopectin into water-soluble pectin. The content of water-soluble pectin in fresh peppers ranges from 1.291 to 1.761 mg/100 g DW [31], while the content of insoluble pectin ranges from 237 to 830 mg/100 g DW.

The anthocyanin content in pickled peppers increased during low-temperature storage and exceeded that in unfrozen pickled peppers by 70.1% at day 270 (**Table 9.3**). The anthocyanin content in fresh pepper fruits depends on the stage of ripeness and, in ripe fruits (purple to red), does not exceed 10 mg/100 g [32].

The catechin content in pickled peppers was 63.2 mg/100 g (**Table 9.3**). During the first 10 days of storage after freezing, it increased slightly to 66.2 mg/100 g. During the subsequent storage period, the catechin content more than doubled. After 270 days of storage, the catechin content of frozen pickled peppers reached 133.1 mg/100 g. For comparison, the catechin content in fresh peppers ranges from 74.5 to 79.4 mg/100 g [33].

The total flavonoid content during storage followed a pattern similar to that of catechin: an initial slight increase followed by a doubling after 90 days (**Table 9.3**). Prior to freezing, the flavonoid content in pickled peppers was 144.6 mg/100 g, and after 270 days of low-temperature storage, it reached 347.7 mg/100 g. For comparison, the total flavonoid content in fresh peppers ranges from 80.0 to 112.6 mg/100 g [34].

9.3.4 Microbiological indicators of frozen pickled sweet peppers

The microorganisms most commonly responsible for food spoilage include *Pseudomonas*, *Enterobacteriaceae*, and *Brochothrix thermosphacta* [37]. These organisms cause slime formation, deterioration of food texture, and the development of off-flavors. During storage, low-temperature conditions inhibit the growth of microorganisms [38]. The quantity of microorganisms in frozen products depends on several factors, including pH, temperature, product type, pre-treatment, storage time before freezing. Due to the risk of microbial growth, pre-treatment operations should be performed as quickly as possible. The spores of many bacteria do not die during prolonged exposure to an acidic environment, although they do not develop under such conditions. A neutral or slightly alkaline environment promotes their longer

survival. The presence of sugar in the solution also helps to preserve their resistance during freezing. Pathogenic microorganisms that cause food poisoning do not develop in quick-frozen products, as they are not psychrophilic and have a minimum growth temperature of 5°C. Some microorganisms are inactivated during freezing, while others are inactivated during storage in the frozen state. The destruction of microflora at low temperatures is slow and depends on the composition and type of microorganisms. Gram-negative bacteria, including coliforms, are more sensitive to low temperatures than Gram-positive bacteria.

Microbiological analysis of fresh sweet pepper samples demonstrated contamination with microorganisms, including molds and bacteria, while no yeasts were detected on the pepper fruit surface (**Table 9.4**).

Table 9.4 Analysis of epiphytic microflora in frozen pickled sweet peppers

Storage (days)	Number of microorganisms (CFU/mm^2)		
	Molds	Yeasts	Bacteria
0 (fresh pepper)	483	n.d.	16,918
10	2.1	n.d.	5,212
90	1.6	n.d.	6,213
180	3.0	n.d.	6,850
270	5.1	n.d.	11,257

Note: n.d. – not detected

After 10 days of storage, the number of microorganisms in frozen pickled peppers decreased sharply compared to fresh peppers. In particular, the number of molds decreased from 483.0 to 2.1 CFU/mm^2, and the number of bacteria decreased from 16,918 to 5,212 CFU/mm^2. No yeasts were detected throughout the entire storage period. These results indicate that molds and certain types of bacteria are resistant to the acidic environment of the marinade (**Table 9.4**). After 90 days of storage, the number of molds further decreased to 1.6 CFU/mm^2, while bacteria increased to 6,213 CFU/mm^2. During prolonged storage, the number of molds and bacteria gradually increased, indicating that they retained the ability to grow. After 270 days of storage, the number of molds reached 5.1 CFU/mm^2 and bacteria 11,257 CFU/mm^2. However, these values did not exceed those observed in fresh peppers, although the number of bacteria increased sharply during the final three months of storage.

Low microorganism content is a key factor in ensuring the microbiological safety of frozen pickled peppers. The microbiological indicators fully comply with sanitary standards and regulations, confirming that the product is safe for consumption.

9.4 Thawed pickled sweet peppers

9.4.1 Effect of different thawing techniques on the sensory properties of pickled sweet peppers

Frozen pickled sweet peppers were thawed using different techniques, including conventional air-thawing at room temperature (20°C), water thawing, and thawing in a microwave oven. All samples of frozen pickled peppers were stored at −20°C for 90 days prior to thawing.

In terms of appearance, the pickled pepper sample thawed using microwave-assisted thawing received the highest score of 5.0 (**Table 9.5**), while the lowest score (4.4) was observed for the air-thawed sample. A similar trend was observed for color. The aroma of peppers thawed using water thawing and microwave-assisted thawing was rated highly, with scores of 4.9 and 5.0, respectively. All thawed samples received high scores for taste, with water-thawed and microwave-thawed samples achieving the maximum score of 5.0. Regarding consistency, the air-thawed and water-thawed samples received lower scores (4.2 and 4.1, respectively) compared to the microwave-thawed sample (4.7). The highest average sensory score (4.94) was observed for peppers thawed using microwave-assisted thawing, indicating that this technique is the most effective for maintaining the sensory quality of pickled sweet peppers.

Table 9.5 Sensory evaluation of thawed pickled sweet peppers (points)

Thawing techniques	Sensory evaluation (mean)					
	Appearance	Color	Aroma	Taste	Consistency	Average score
Air-thawing	4.4	4.6	4.5	4.8	4.2	4.50
Water thawing	4.7	4.0	4.9	5.0	4.1	4.54
Microwave-assisted thawing	5.0	5.0	5.0	5.0	4.7	4.94

9.4.2 Ascorbic acid content of thawed pickled sweet peppers

Table 9.6 presents the ascorbic acid content in microwave-thawed pickled pepper samples after storage in air at room temperature (20°C) for 2, 4, 6, and 10 hours. These measurements were performed on frozen pickled peppers after 10, 90, 180, and 270 days of low-temperature storage.

The ascorbic acid content in thawed pickled peppers after two hours of storage at room temperature decreased by 5.6–11.1% compared to its level immediately after thawing (**Table 9.6**). Samples stored in a frozen state for a longer period exhibited a higher percentage of ascorbic acid loss. A similar trend was observed in samples stored for 4, 6, and 10 hours after thawing. Compared to the content immediately after thawing, the ascorbic acid level decreased by 9.2–13.1% after 4 hours of storage, by 11.6–16.1% after 6 hours, and by 22.6–24.8% after 10 hours. In absolute terms, the ascorbic acid content decreased both with increasing duration of frozen storage and with prolonged storage in the thawed state. The lowest ascorbic acid content (136.8 mg/100 g) was observed in the sample stored at low temperatures for 270 days and subsequently held at room temperature for 10 hours after thawing.

Table 9.6 Ascorbic acid content of thawed pickled sweet peppers during short-term storage (mg/100 g)

Storage of thawed-samples (hours)	Frozen storage (days)			
	10	90	180	270
0	199.6	192.4	187.3	181.8
2	187.8	175.6	169.2	161.6
preserved in peppers (%)	94.1	91.3	90.3	88.9
4	181.2	170.3	163.7	157.9
preserved in peppers (%)	90.8	88.5	87.4	86.9
6	176.5	163.5	157.9	152.6
preserved in peppers (%)	88.4	85.0	84.3	83.9
10	154.5	148.5	141.1	136.8
preserved in peppers (%)	77.4	77.2	75.3	75.2

9.4.3 Enzyme activity in thawed pickled sweet peppers

The dynamics of enzymatic changes in frozen foods are determined by their chemical composition, properties, and storage conditions. Subzero temperatures do not cause complete or long-term enzyme inactivation, but rather lead to temporary and partial inhibition of enzyme activity due to changes in environmental conditions, including a reduction in the liquid phase resulting from water crystallization, an increase in ion concentration, and changes in the pH of the medium. The quality of sweet peppers may be affected by enzymatic activities. In particular, polyphenol oxidase catalyzes the hydroxylation of monophenols to *o*-diphenols and the oxidation

of *o*-diphenols to *o*-quinones [39]. Further reactions of these quinones result in the accumulation of melanin, which is responsible for browning in plant tissues.

Polyphenol oxidase activity in pepper fruits was determined during the storage period, beginning immediately after thawing and continuing after 2, 6, 12, and 24 hours of storage in the thawed state. The results of the polyphenol oxidase activity determination are presented in **Table 9.7**. In the sample of pickled pepper thawed immediately after freezing, polyphenol oxidase activity was 5.0 μmol/min. During storage of the thawed sample at room temperature (20°C), enzyme activity increased, reaching 17.58 μmol/min after 12 h and then slightly decreased to 15.98 μmol/min after 24 h. Thus, after 24 h of storage in the thawed state, polyphenol oxidase activity was approximately three times higher than immediately after thawing. A similar pattern of changes was observed in samples thawed on days 90, 180, and 270 of low-temperature storage. However, the initial enzyme activity decreased with increasing duration of frozen storage. In particular, the polyphenol oxidase activity was 4.75 μmol/min in the sample thawed on day 90, 4.12 μmol/min in the sample thawed on day 180, and 4.01 μmol/min in the sample thawed on day 270. The highest polyphenol oxidase activity in all samples was observed 12 h after thawing: 16.31 μmol/min after 90 days of frozen storage, 14.98 μmol/min after 180 days, and 13.65 μmol/min after 270 days.

Table 9.7 Polyphenol oxidase activity in thawed pickled sweet peppers during short-term storage (μmol/min)

Frozen storage (days)	Storage of thawed-samples (hours)				
	0	2	6	12	24
0 (immediately after freezing)	5.00	13.68	16.67	17.58	15.98
90	4.75	12.03	15.11	16.31	13.72
180	4.12	10.87	13.02	14.98	11.23
270	4.01	10.11	12.18	13.65	10.98

9.4.4 Microbiological indicators of thawed pickled sweet peppers

Freezing does not completely eliminate microorganisms, and a large number of bacterial spores remain viable. After thawing, these spores may germinate and cause rapid spoilage of the product. Thawed products also provide a favorable nutrient medium for the growth of various microorganisms.

During the thawing of quick-frozen products, psychrophilic microorganisms are the first to multiply. Their metabolic activity begins long before the temperature becomes sufficiently high for the growth of pathogenic microorganisms. Therefore, even after a relatively long period following thawing, products frozen without prior heat treatment are generally not a source of infection by pathogenic microorganisms. However, microorganisms that are harmless to human health but cause food spoilage may proliferate, leading to product deterioration and making the food unfit for consumption. Foods manufactured in accordance with technological requirements for pre-treatment, freezing, storage, and thawing are considered safe according to hygienic standards.

Immediately after thawing, the frozen pickled pepper sample stored at low temperature for 10 days contained 5,212 CFU/mm^2 of bacteria and 2.1 CFU/mm^2 of mold (**Table 9.8**). No yeast was detected in the sample. During the first 12 h of storage at room temperature (20°C), bacterial and mold counts increased to 12,380 CFU/mm^2 and 10.4 CFU/mm^2, respectively. This increase can be explained by the rise in ambient temperature and product moisture after thawing, which creates favorable conditions for the intensive growth of microorganisms. During the following 12 h, microbial counts decreased, with bacteria dropping to 7,430 CFU/mm^2 and molds to 8.3 CFU/mm^2. This decline is likely due to microbial competition, the accumulation of metabolic by-products, and environmental changes that inhibit microbial activity. Yeasts remained undetected throughout the entire storage period at room temperature.

Table 9.8 Analysis of the number of microorganisms in thawed pickled sweet peppers (CFU/mm^2)

Frozen storage (days)	Microorganisms	Storage of thawed-samples (hours)				
		0	2	6	12	24
10	Bacteria	5,212	6,920	10,815	12,380	7,430
	Yeasts	n.d.	n.d.	n.d.	n.d.	n.d.
	Molds	2.1	3.8	7.5	10.4	8.3
90	Bacteria	6,213	9,200	13,500	15,980	11,300
	Yeasts	n.d.	n.d.	n.d.	n.d.	n.d.
	Molds	1.6	2.0	4.1	8.0	6.0
180	Bacteria	6,850	18,308	25,660	48,400	23,170
	Yeasts	n.d.	n.d.	n.d.	n.d.	n.d.
	Molds	3.0	5.0	8.0	12.0	9.0
270	Bacteria	11,257	13,100	18,600	21,430	14,123
	Yeasts	n.d.	n.d.	n.d.	n.d.	n.d.
	Molds	5.1	9.0	15.0	19.0	14.0

Note: n.d. – not detected

A similar pattern of microbial growth was observed in pickled sweet pepper samples thawed after 90, 180, and 270 days of frozen storage. In the sample frozen for 90 days, bacterial and mold counts immediately after thawing were 6,213 CFU/mm^2 and 1.6 CFU/mm^2, respectively. After 12 h at room temperature, these counts increased to 15,980 CFU/mm^2 for bacteria and 8.0 CFU/mm^2 for molds. After 24 h of thawed storage, the counts decreased to 11,300 CFU/mm^2 for bacteria and 6.0 CFU/mm^2 for molds.

Analysis of the microbiological state of thawed pickled pepper samples indicated that longer frozen storage led to higher microbial counts after thawing. Under favorable conditions, microorganism multiplied actively during the first 12 h of thawed storage. The highest counts of bacteria and molds were observed in samples thawed after 180 and 270 days of low-temperature storage and kept at room temperature for 12 h. In particular, the highest bacterial count (48,400 CFU/mm^2) was recorded after 12 h of room-temperature storage of a sample previously frozen for 180 days. The maximum mold count (19.0 CFU/mm^2) was observed after 12 h of room-temperature storage of a sample frozen for 270 days.

Thus, after thawing, the bacterial and mold counts in pickled peppers, even after 24 h of storage at 20°C, remain below the permissible levels for quick-frozen vegetables: bacteria – $7 \cdot 10^4$ CFU/g, molds – $1 \cdot 10^2$ CFU/g, and yeasts – $5 \cdot 10^2$ CFU/g. However, storing thawed pickled peppers at 20°C is not recommended, even in the absence of toxigenic or pathogenic microorganisms. Thawed pickled peppers should be stored only for the time required for thawing and are best consumed immediately after thawing.

9.5 Conclusions

The scientific novelty of this study lies in the comprehensive assessment of the quality and safety of frozen pickled sweet peppers during low-temperature storage and in determining the effects of different thawing techniques and short-term storage of the thawed product on its quality attributes.

Quick freezing of sweet peppers ensures the preservation of their sensory and nutritional properties for a long period. Pickling sweet peppers before freezing makes it possible to obtain a product that is ready for consumption immediately after thawing. During low-temperature storage of pickled peppers, the content of ascorbic acid is maintained at 80.2–88.0% and carotenoids at 84.9–94.2% of the initial values, while the contents of water-soluble pectin, anthocyanins, catechins, and total flavonoids increases. The contents of dry matter and total sugars in frozen

peppers decrease slightly during storage by 0.7–4.9% and 0.6–6.1%, respectively. The amount of epiphytic microflora in frozen pickled sweet peppers is lower compared to that in fresh sweet peppers. As a result, the sensory properties of the product remain highly rated even after 270 days of frozen storage.

The most appropriate technique for thawing pickled sweet peppers is microwave thawing, as it ensures high sensory quality of the product, particularly in terms of consistency. This technique is also considerably faster than thawing in water or air. When thawed pickled pepper are stored at 20°C for up to 10 h, the ascorbic acid content decreases by up to 25%, and polyphenol oxidase activity increases, although the microbiological safety of the product remains ensured. However, it is recommended that thawed sweet peppers be consumed immediately after microwave thawing.

Further studies are needed to determine optimal packaging materials and conditions for frozen pickled peppers in order to preserve their quality during storage and transportation and ensure the product's safety for consumers.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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The authors confirm that they did not use artificial intelligence technologies when creating the current work.

Authors' contributions

Nadiia Zahorko: Conceptualization, Supervision, Methodology, Empirical research design, Data analysis, Writing – original draft.

Igor Dudarev: Conceptualization, Literature review, Methodology, Data analysis, Writing – original draft.

Valentyna Tkachuk: Literature review, Interpretation of results, Validation, Language editing, Academic writing support, Writing – review & editing.

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CHAPTER 10

Effect of a combined biopolymer coating on the quality of asparagus spears during storage

Olesia Priss
Liudmyla Kiurcheva
Pavlo Bulhakov
Iryna Taraymovich
Larysa Chepurda
Sergii Stepanenko

Abstract

The chapter is devoted to the study of the influence of a biopolymer coating based on sodium alginate and the antioxidant rutin on the quality of asparagus spears of Prius and Rosalie varieties during storage under refrigerated conditions.

The influence of the coating on the preservation of marketable, physiological and organoleptic characteristics of asparagus was considered.

It was found that the most pronounced effect was obtained when using a combined coating (1% sodium alginate +1% rutin), which combines the barrier properties of the biopolymer and the antioxidant activity of rutin.

The use of such a composition allows to extend the shelf life of spears of both varieties by 7 days compared to the control, reduce mass loss by 1.9 times for the Prius variety and by 2.2 times for the Rosalie variety and increase the yield of standard products to 88.14–91.79% depending on the variety, despite the increased storage time.

The use of the studied biopolymer made it possible to slow down respiratory metabolism, degradation of chlorophylls and carotenoids and stabilize the organoleptic characteristics of the product.

Keywords

Asparagus, biopolymer coating, sodium alginate, rutin, weight loss, yield of marketable products, pigments, respiratory rate, postharvest treatment.

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10.1 Introduction

Modern world vegetable growing is developing in conditions of aggravation of food security. This fact emphasizes the importance of developing technologies for the production and storage of fruit and vegetable products that minimize their losses at all stages of the logistics chain connecting the producer and the end consumer. This is especially important for crops that have high market potential, but are characterized by limited shelf life. Asparagus (*Asparagus officinalis* L.), which is characterized by high biological value and growing demand among consumers, has good prospects in this aspect. Asparagus belongs to valuable vegetable crops, characterized by high taste properties and significant nutritional value. The crop is gradually gaining popularity due to trends in expanding the range of plant crops in human nutrition and the globalization of gastronomic preferences. Despite the availability of processed products (canned, quick-frozen), the main direction of asparagus use remains fresh sale. The active growth in demand for asparagus, especially in the HoReCa segment, as well as its relatively high profitability, also cause increased interest among agricultural producers in expanding the area under this crop [1]. At the same time, the economic efficiency of its production largely depends not only on the yield, but also on the ability to preserve the marketable quality of the product in the post-harvest period. This places increased demands on the preservation of its natural properties during transportation and refrigerated storage. Therefore, the segment of fresh asparagus production requires the improvement of technological solutions aimed at minimizing post-harvest losses and extending the period of sale without deterioration in quality.

The season for the sale of fresh asparagus in Ukraine is limited and lasts from the end of April to the beginning of June, which additionally makes the issue of extending its storage period relevant. Asparagus spears 15–22 cm long and up to 2 cm thick are used in food. The plant is in a phase of active growth and, accordingly, with a particularly active tissue metabolism. Asparagus spears are extremely sensitive to the conditions of the post-harvest period, which is due to the high intensity of respiration, intensive gas exchange and transpiration. This leads to rapid weight loss, a decrease in turgidity, deterioration of the texture and general marketable appearance of the product. After cutting, the respiratory rate increases even more due to wound stress, which is accompanied by increased transpiration and biochemical transformations [2]. As a result, the harvested spears extremely quickly lose mass, turgidity, consumer properties, and their nutritional value decreases. At the same time, the amount of waste, non-marketable products that cannot be sold, increases. Therefore, extending the storage period of asparagus and minimizing quantitative and qualitative losses in the post-harvest period have not only economic, but also

socio-ecological significance. Reducing waste volumes will contribute to increasing the profitability of production, stabilizing supplies to the market and extending the period of consumption of fresh products. In addition, preserving the marketable quality and nutritional value of asparagus during storage ensures greater accessibility of this functionally valuable vegetable for consumers, which has a positive effect on the formation of balanced diets [3].

Therefore, the above makes it necessary to find effective technological solutions aimed at slowing down physiological processes, reducing mass losses and preserving the quality of asparagus for a longer period of sale.

10.2 Changes in asparagus quality during storage

Product losses during storage and a decrease in quality indicators are caused by a number of factors, including natural (loss of mass, overripening, aging, etc.), phytopathological (disease damage), technological (non-compliance with storage conditions), mechanical (damage to products during transportation and primary processing), which significantly accelerate degradation processes.

The main goal of effective product storage is to ensure conditions for its vital activity for a long period, inhibit the processes of overripening and aging without reducing marketable quality while maintaining sufficient resistance to microbiological and functional diseases. Extending the period of receipt of fresh vegetables is achieved mainly by refrigerated storage, sometimes in combination with other methods aimed at slowing down the metabolism of vegetables [4].

During refrigerated storage, however, microbiological, biochemical and physical transformations occur in fresh plant products, which cause changes in their quality and marketability. Asparagus spears are characterized by a high metabolic rate, which leads to accelerated overripening and aging processes, and accordingly, limits the duration of storage. External quality indicators, such as the diameter and color of the spears, the shape of the top and the peculiarity of the fit of the leaf scales, are considered the main ones for assessing the commercial quality according to the requirements of the standards. The group of organoleptic indicators (consistency of the pulp, taste, level of bitterness) is a priority for consumers. During storage, the spears discolor as a result of the decomposition of chlorophylls, the loss of nutrients and organic acids, the accumulation of asparagine, and changes in texture. The latter is due to lignification and an increase in fiber content (by 72% according to [4]). The fit of the leaf scales also changes significantly during storage of some varieties (due to loose fit to the spear, the so-called "pluminess" develops).

Moisture evaporation is a natural process caused by water migration from asparagus spear tissues. Due to their relatively rapid turgidity loss, asparagus spears become wilted and more susceptible to physiological disorders and microbiological diseases. The intensity of water loss depends on the hydrophilicity of cellular colloids, as well as on the structure and properties of the protective tissues. Spear sections with a larger surface area-to-mass ratio exhibit higher moisture loss rates, as do shorter spears [5].

The dynamics of asparagus spears aging are influenced by their structural heterogeneity: the bud contains parenchymal meristematic cells, which active division drives spear growth. These cells are small, densely packed, have thin walls composed of hemicellulose and pectin compounds, and exhibit high metabolic rates, requiring a constant supply of water and nutrients. In contrast, the lower parts of the spears consist primarily of mature tissue where cell elongation has ceased. Respiratory activity in the buds is high (about 60 mg $CO_2 \times kg^{-1} \times h^{-1}$ at 5°C), exceeding that in the lower spear parts by an average of 4 times at the start of storage and by 2 times after 3 weeks [6]. Immediately after harvest, respiratory processes slow down and stabilizing after 12–24 hours.

Pigments are an important visual indicator used to assess the quality of asparagus spears. The chlorophyll content of green spears varies depending on the part of the spears, ranging from about 10 mg/ml in the upper part to about 8 mg/ml in the middle. During storage, chlorophyll levels gradually decrease, accompanied by yellowing of the spears. Several factors can slow down the degradation of chlorophyll during storage, including low temperature, the use of polypropylene packaging, elevated CO_2 levels, and treatment with a cholesterol solution [7]. Color changes in white and purple asparagus varieties are mainly due to the gradual synthesis and accumulation of flavones (which cause yellowing) and anthocyanins (which cause reddening).

Understanding the nature of the changes that occur in asparagus spears after harvest and initiate the aging process allows to predict the quality of asparagus during storage and manage technological regimes to improve quality indicators.

10.3 Asparagus storage technologies

At room temperature, the storage life of asparagus averages only 3–5 days, whereas under refrigerated conditions it extends to approximately 14–15 days [8]. Refrigerated storage is the standard method for preserving fruits and vegetables, as it significantly reduces the respiratory metabolism of plant products. However, under conventional cold storage conditions, lignification processes in asparagus

spears also begin relatively quickly. To slow these processes, refrigeration should be combined with additional methods of preliminary chemical or physical treatment.

Postharvest heat treatment prior to storage involves exposing the produce to elevated temperatures (immersion in hot water, heating with saturated steam, or treatment with hot dry air). According to a study conducted by Taiwanese researchers [9], preliminary hot-water treatment of asparagus (immersion in water at 48°C for 4 min) increases storage efficiency both when applied alone and when combined with subsequent refrigerated storage and film packaging. Currently, postharvest heat treatment is used mainly for the storage of organic produce.

To inhibit vegetable ripening processes, hypobaric storage – storage under reduced atmospheric pressure – is increasingly applied. According to literature data [10], hypobaric storage of asparagus significantly suppresses the respiratory activity of the spears, promotes the preservation of chlorophylls, antioxidants, and soluble solids, and reduces the accumulation of malondialdehyde. As a result, overripening processes are delayed, allowing the storage period to be extended up to 50 days. However, this storage method requires expensive specialized equipment.

To extend the shelf life of asparagus, refrigerated storage is often supplemented by the use of various films, vacuum packaging, and modified atmosphere packaging (MAP) [11]. The use of synthetic polymer materials to maintain modified atmosphere conditions during storage has been shown to be highly effective. However, one drawback of MAP storage is the accumulation of excessive condensate on the inner surface of the packaging, which stimulates the development of microflora and leads to premature spoilage of the product.

Nevertheless, the large volumes of waste generated after the use of polymer packaging materials worsen the global environmental situation, which stimulates the search for more environmentally friendly alternatives.

To preserve the post-harvest quality of asparagus, treatment of spears with 1-methylcyclopropene (1-MCP), which is an ethylene inhibitor and is able to delay the ripening and senescence processes, is widely used. Treatment with a 4 ml/l solution of 1-MCP effectively delays the lignification process when asparagus is stored at 4°C and 80% relative humidity, allowing to extend the storage period up to 37 days [12]. The decrease in ethylene concentration in 1-MCP-treated samples was also confirmed in [13], although this study did not reveal a significant effect of 1-MCP on the quality and preservation of asparagus spears under MAP conditions and in perforated film.

Given the growing demand for the use of environmentally friendly and safe substances for human health for post-harvest processing, research into the development of edible food coatings is becoming increasingly relevant. It has been shown

that such coatings extend the shelf life and maintain the quality of various fresh vegetables, such as carrots, potatoes, eggplants, tomatoes, and bell peppers [14].

Edible coatings are thin external layers applied to the surface of fresh fruits and vegetables to improve their appearance, reinforce the natural waxy cuticle, minimize moisture loss during storage, protect against mechanical damage, and create an individual modified atmosphere. Edible coatings have several advantages, including ease of application, energy efficiency, and scalability of production, as well as proven safety (classified as GRAS by the FDA) due to their origin from food-grade materials [15].

Edible coatings act as barriers to gases and moisture, thereby not only limiting respiratory metabolism but also enhancing the antioxidant properties of the produce by restricting oxygen access. In addition, they serve as carriers of functional or biologically active compounds incorporated into the formulation to preserve or improve product quality.

Polysaccharides such as chitosan, sodium alginate, carboxymethylcellulose, and pectins have good film-forming properties, thus demonstrating the potential for use as food coatings. Forming a dense framework, polysaccharide films completely cover the fruits and effectively delay weight loss, a decrease in the content of anthocyanins, and secondary metabolites [16]. Alginates are hydrophilic colloidal carbohydrates extracted from various species of brown seaweeds belonging to the class Phaeophyceae. Films formed with sodium alginate are uniform, transparent, and act as good oxygen barriers, but due to their hydrophilic nature, they are not waterproof [17]. Glycerol is used to improve the plasticity of alginate films, which improves the flexibility of the film but increases the permeability of water vapor [18]. Sodium alginate-based films can help maintain the quality of fruits during storage, especially in combination with antioxidants [19]. Alginate films successfully act as carriers of bioactive substances. Phenolic compounds are often used as antioxidants. However, these substances sometimes diffuse into food products, imparting undesirable taste and aroma due to the presence of a mixture of volatile and non-volatile components, which limits their application [16]. The use of many polyphenols (quercetin, other flavonoids) in alginate films has been described, emphasizing that such a strategy is universal for flavonoid antioxidants [20]. Rutin, which is contained in asparagus in significant quantities and, when used exogenously, will not change the taste and aroma of the product, also belongs to this group. It can be assumed that the introduction of the natural antioxidant rutin into the alginate composition will allow extending the shelf life of asparagus processed in this way. Therefore, the aim of the work was to confirm the possibility of extending the shelf life of asparagus spears and stabilizing their quality indicators by using a combined biopolymer coating based on alginate and rutin.

10.4 Research methodology

In the study, asparagus spears (*Asparagus officinalis* L.) of two hybrids with different coloration were used – Prius F1 (green) and Rosalie F1 (purple-green). For the experiment, uniform, straight, and undamaged spears with a diameter of 1.6–2.0 cm and a length of approximately 25 cm were selected. The spears had closed bracts and showed no signs of wilting or mechanical damage, in accordance with the requirements of the standard for fresh asparagus CODEX STAN 225-2001, which defines the criteria for sizing, appearance, freshness, and permissible defects of the product (**Fig. 10.1**).

a *b*

Fig. 10.1 Asparagus variety Prius: *a* – calibrated; *b* – after commercial processing

The research program involved a stepwise substantiation of the combined biopolymer coating composition. Initially, the effects of sodium alginate biopolymer (A) and the sodium alginate-glycerol combination (A + G) were evaluated on asparagus storage duration, natural mass loss during storage, and organoleptic indicators.

Asparagus spears (of both varieties) were treated with the following biopolymer coating variants:

– A – 1% aqueous sodium alginate solution (dry substance gradually dissolved in hot water at ($t = 45$°C));

– G – 1% aqueous glycerol solution;

– A + G – 1% aqueous sodium alginate solution combined with 1% glycerol solution;

– C – untreated (control).

Parallel evaluations assessed the antioxidant rutin's (R) effects on the same indicators. Asparagus spears were treated with aqueous rutin solutions at concentrations of: 0.5% (R0.5), 1% (R1), 1.5% (R1.5) or left untreated (C, control). Rutin solutions were prepared by dissolving dry powder in 96% ethanol (5% by mass) and diluting to the required concentration with water.

For coating application, asparagus spears were fully immersed in cooled solutions. After removal, spears were placed vertically on a rack over a drip tray to drain excess solution and air-dry for 1 hour under cooling conditions. Treated and untreated samples were then placed in refrigerated storage. Storage was considered complete when losses and waste reached 10%, with waste including rotten produce and items showing microbial damage signs. Marketable and physiological indicators of asparagus spears were assessed at the start and end of storage. Storage durations for experimental samples were extended relative to controls until spear damage appeared.

At the next stage, the effectiveness of the combined coating was investigated. Asparagus spears were treated with the combined biopolymer coating A + R – a 1% aqueous sodium alginate solution combined with a 1% rutin solution (alginate was added to the preheated 1% rutin solution and left for cooling and uniform gel formation for 20 min). Coating application and subsequent storage followed the scheme described above.

Organoleptic characteristics were assessed according to the following parameters: turgidity (from a fresh appearance to severe loss of turgidity), longitudinal striation (from absence of striations to pronounced striation), desiccation of the bases (from no desiccation to severe desiccation), color changes (from bright green or purple-green typical for the variety to yellowing), off-odors (from absence of odors to noticeable off-odors), and microorganism spoilage (from absence of visible microbial damage to clear signs of spoilage).

A four-point scale was used for evaluation: 4 – very good; 3 – good; 2 – acceptable; 1 – unacceptable.

In addition, an importance coefficient was introduced for the overall assessment, considering the critical influence of each parameter on consumer satisfaction: 0.3 for turgidity; 0.2 for off-odors and microorganism spoilage; and 0.1 for the other indicators.

To determine the effect of biopolymer coatings on respiratory metabolism, the respiration rate was assessed by measuring the absorption of carbon dioxide (CO_2) by an alkali solution [21]. The chlorophyll and carotenoid contents were determined by extracting the pigments with acetone followed by spectrophotometric analysis [22].

The experiments were carried out with replication in accordance with the applied methodology. Data were processed using standard statistical methods, with calculation of mean values and standard deviations.

10.5 Effect of biopolymer coating on the preservation of asparagus quality during storage

Control samples of both varieties stored in refrigerated conditions maintained acceptable quality for no more than 14 days. Further storage was accompanied by yellowing of the spears, loss of firmness and lignification of tissues, which was confirmed by the results of organoleptic evaluation (**Fig. 10.2**).

In this case, the Prius variety demonstrated a higher number of non-marketable products after 14 days of storage (**Fig. 10.3**).

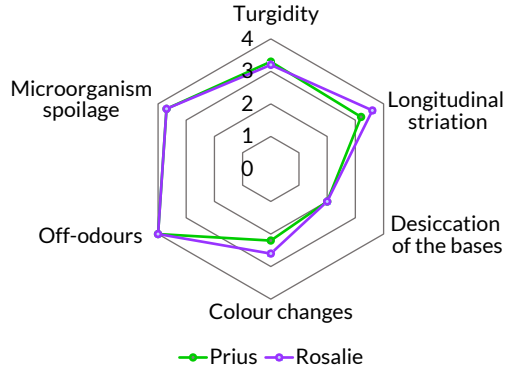

Fig. 10.2 Organoleptic evaluation of raw asparagus spears after 14 days of storage

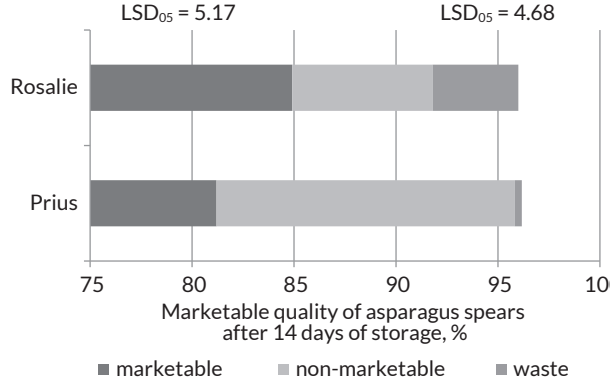

Fig. 10.3 Marketability of asparagus spears after 14 days of storage. The indicators are presented taking into account natural weight losses

This can be explained by the greater tendency of green spears to open bracts during storage, which is one of the key parameters for determining marketable quality. As can be seen from **Table 10.1**, the use of biopolymer coatings allowed to extend the shelf life of asparagus to 18 days compared to control samples (14 days).

Table 10.1 Marketable quality of asparagus spears after storage with biopolymer coatings, %, $M \pm m$, $n = 5$

Variety	Treatment	Storage time, days	Products, %		
			marketable	non-marketable	waste
Prius	A	18	88.69 ± 1.57*	5.85 ± 0.80*	2.97 ± 0.92*
	G	14	82.25 ± 0.63	10.55 ± 0.42	3.35 ± 0.39*
	A + G	18	84.32 ± 1.29*	7.93 ± 0.96*	4.07 ± 0.88*
	C	14	81.20 ± 1.16	14.62 ± 0.98	0.35 ± 0.19
Rosalie	A	18	88.14 ± 0.82*	4.98 ± 0.38*	3.91 ± 0.53
	G	14	84.48 ± 0.99	6.23 ± 0.51	2.15 ± 0.47
	A + G	18	85.72 ± 1.25	4.74 ± 0.66*	2.30 ± 0.45
	C	14	84.91 ± 1.53	6.89 ± 0.41	4.20 ± 1.64

*Note: marketable indicators were calculated at the end of storage, taking into account natural weight losses. * – significant difference compared with the control on the day of measurement ($p \leq 0.05$)*

This effect was observed for both varieties, although the indicators of product losses and the amount of marketable products differed somewhat between Prius and Rosalie. The obtained data are consistent with observations [22], which indicated a reduction in mass losses and an extension of the storage period of spears treated with compositions based on alginate, chitosan and carrageenan by 3 days compared to control samples.

Treatment with alginate reduced weight loss by 1.4 times compared with the control and improved the yield of marketable produce. A decrease in the number of non-marketable spears was also observed, especially noticeable in the example of the Prius variety, which indicates the stabilizing effect of biopolymer coatings on the marketable quality of asparagus during storage.

Additionally, the experimental samples showed a larger area of the profilogram compared to the controls in terms of organoleptic indicators (**Fig. 10.4**).

The most noticeable positive effect of the treatment was on such indicators as turgidity and color changes. Maintaining turgidity at a constant level for a longer time, which also correlates with a decrease in mass loss, seems natural given the expected effect of the coating on the intensity of respiration and transpiration due to the formation of a film over the stomata. The improvement in the color index may also be associated with the slowing down of chlorophyll decomposition in conditions

of a general slowdown in metabolism. To improve the plasticizing properties of the coating, the possibility of introducing glycerol into the composition of the biopolymer was investigated. The introduction of glycerol into the sodium alginate solution (A + G), although it contributed to the formation of films of more uniform thickness, did not significantly affect the marketable quality of the samples and their organoleptic indicators compared to treatment with a separate sodium alginate solution. The control treatment of spears with glycerol also did not have a significant effect on the quality of asparagus. In the case of the Rosalie variety, weight losses during such treatment were even higher than in untreated samples. Given the lack of a positive effect of mixtures with glycerol on the dynamics of asparagus respiration intensity (**Table 10.2**), the inclusion of glycerol was not further considered as a promising option for post-harvest treatment of asparagus.

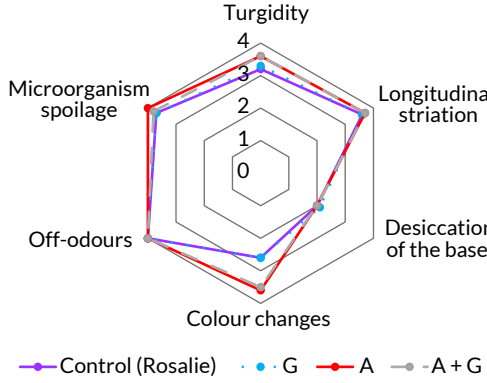

Fig. 10.4 Organoleptic evaluation of asparagus at the end of storage after treatment with biopolymer coatings: *a* – Prius; *b* – Rosalie

Table 10.2 Respiration rate (mgCO_2 × kg^{-1} × h^{-1}) of asparagus treated with glycerol-based compositions

Day of storage	Rosalie				Prius			
	Control	G	A + G	LSD_{05}	Control	G	A + G	LSD_{05}
0	106.2	106.2	106.2	–	94.9	94.9	94.9	–
1	65.2	118.6	167.3	17.99	55.4	65.9	59.5	10.82
7	94.6	83.0	134.2	25.30	81.4	98.6	80.0	13.47
12	98.2	114.2	83.9	14.25	94.5	89.9	78.6	12.01
21	117.7	85.7	69.9	12.62	128.4	52.2	71.0	10.60
37	–	–	–	–	80.0	92.6	121.6	17.42

Given that the main action of biopolymer coatings is primarily aimed at limiting physical moisture losses from plant raw materials, for effective inhibition of post-harvest metabolism it is advisable to use biologically active compounds with antioxidant properties, which are able to additionally stabilize cell structures and slow down oxidative processes.

10.6 The effect of rutin on the marketable quality and organoleptic characteristics of asparagus spears

Rutin, which is naturally found in asparagus in significant quantities, is a powerful antioxidant and participates in processes related to pigment metabolism. This makes it advisable to use it as a functional component of a coating for processing spears. Our previous studies prove the high efficiency of rutin in chitosan-based coatings for extending shelf life [23]. However, it is necessary to determine its optimal concentration for post-harvest processing of asparagus.

The results obtained indicate that the use of rutin (R) solutions in low concentrations, similar to biopolymer coatings, contributes to the extension of the shelf life of asparagus spears compared to untreated control samples (**Table 10.3**).

Table 10.3 Marketable quality of asparagus spears after storage with rutin treatment, %, $M \pm m$, $n = 5$

Variety	Treatment	Storage time, days	Products, %		
			marketable	non-marketable	waste
Prius	R0.5	18	87.98 ± 1.48*	6.17 ± 0.54*	2.49 ± 0.76*
	R1	18	88.00 ± 1.31*	5.27 ± 0.32*	2.69 ± 1.05*
	R1.5	18	88.5 ± 1.28*	5.11 ± 0.46*	3.18 ± 0.89*
	Control	14	81.20 ± 1.16	14.62 ± 0.98	0.35 ± 0.19
Rosalie	R0.5	18	86.38 ± 0.86	4.79 ± 0.68*	6.10 ± 1.31
	R1	18	87.14 ± 0.78	4.24 ± 0.52*	5.2 ± 1.18
	R1.5	18	87.26 ± 1.32	4.68 ± 0.97*	4.98 ± 0.44
	Control	14	84.91 ± 1.53	6.89 ± 0.41	4.20 ± 1.64

*Note: marketable indicators were calculated at the end of storage, taking into account natural weight losses. * – significant difference compared with the control on the day of measurement ($p \leq 0.05$)*

In control samples, the proportion of standard-quality products ranged from 81.2–84.9% depending on the variety, whereas rutin treatment increased this

to 87.2–88.5%. The Rosalie variety proved more responsive to rutin than Prius, showing both greater increases in standard product yield and relatively lower mass loss during storage.

Analysis of organoleptic characteristics (**Fig. 10.5**) revealed rutin's most pronounced effects on maintaining spears and color intensity. Treatments with 1% and 1.5% rutin solutions significantly delayed turgidity loss even during extended storage, while the 0.5% solution provided benefits but did not achieve maximum quality preservation.

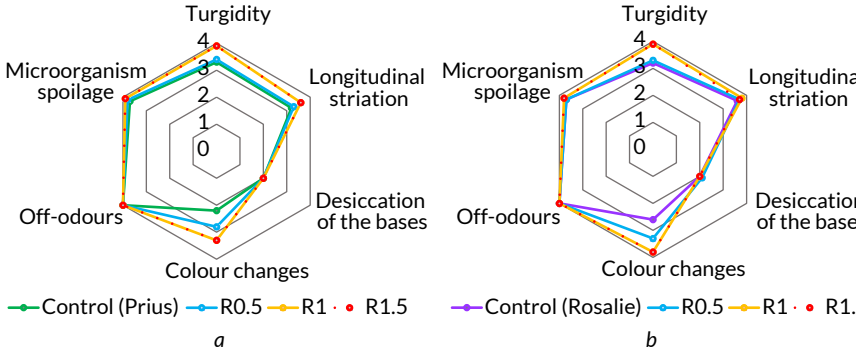

Fig. 10.5 Organoleptic evaluation of asparagus at the end of storage when treated with rutin: *a* – Prius; *b* – Rosalie

Significant differences were also observed in the dynamics of color changes. In the control samples of the Prius variety, the color score decreased to 2.2 points by the end of storage, whereas treatment with rutin allowed this parameter to be maintained at 2.8–3.3 points, depending on the concentration used. For the Rosalie variety, where the control value was 2.6 points, rutin treatment increased this parameter to 3.3–3.8 points by the end of storage.

The positive effect can be explained by the antioxidant properties of rutin, which contribute to the stabilization of plant pigments – primarily chlorophylls and carotenoids – and slow down their oxidative degradation. This likely results in a slower loss of the characteristic color of asparagus spears.

The results of determining the chlorophyll content (**Fig. 10.6**) and carotenoid content (**Fig. 10.7**) at the beginning and at the end of storage confirm this trend. In particular, the application of 1% and 1.5% rutin solutions made it possible to maintain chlorophyll levels 1.8–2 times higher (depending on the variety) compared with the control samples.

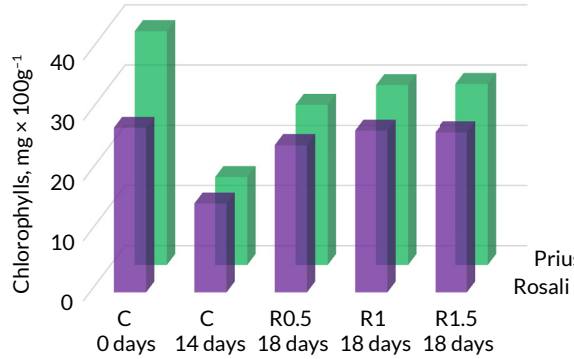

Fig. 10.6 Chlorophyll content in asparagus spears treated with rutin

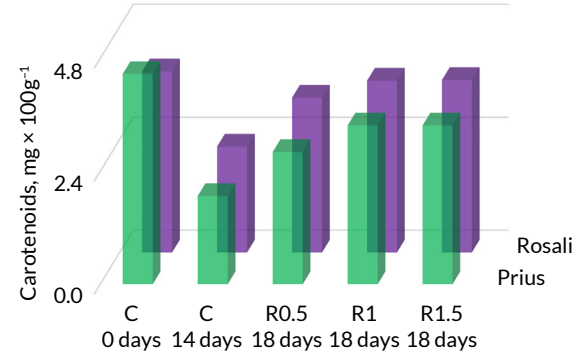

Fig. 10.7 Carotenoid content in asparagus spears treated with rutin

Similarly, carotenoids in samples treated with 1% and 1.5% rutin solutions were preserved 1.6–1.8 times better than in untreated samples.

According to the obtained data, treatment with a 1.5% rutin solution did not result in a significant improvement in the marketable and organoleptic quality parameters of asparagus compared with treatment with a 1% solution, and maintained pigment content at a level similar to that observed for the 1% treatment. At the same time, treatment of asparagus (regardless of variety) with 1% and 1.5% rutin solutions produced better results than treatment with a 0.5% solution. Thus, for further investigation of synergistic effects in the combined coating, a 1% rutin concentration was selected as optimal.

10.7 Effect of the combined biopolymer coating on the marketable quality and organoleptic characteristics of asparagus spears

Based on the results of the previous stage of the study, a composition based on sodium alginate with the addition of 1% rutin (A + R) was tested for a more detailed evaluation of the effect of biopolymer coatings on asparagus storage. The inclusion of rutin in the coating composition provided an additional positive effect regardless of variety, manifested in an extension of storage duration by 7 days compared with the control and by 4 days compared with treatments using sodium alginate alone. After storage, the yield of marketable produce in samples treated with the alginate-rutin composition ranged from 88.14 to 91.79%, depending on the variety, despite the extended storage period (**Fig. 10.8**).

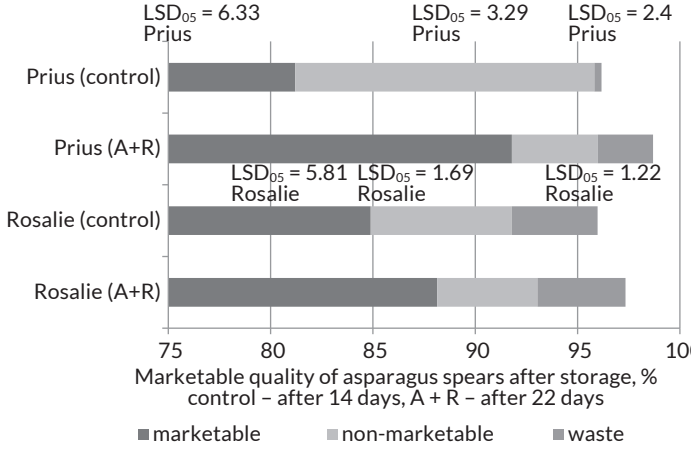

Fig. 10.8 Marketable quality of asparagus spears treated with a combined coating. The indicators are presented taking into account natural weight losses

In addition, these samples showed a reduction in the proportion of non-marketable produce, while the amount of waste under prolonged storage conditions did not increase statistically significantly, indicating the high efficiency of the combined coating.

Regarding organoleptic characteristics, all experimental samples, similarly to the effect observed for simple coatings, demonstrated a larger profilogram area compared with the controls (**Fig. 10.9**).

The application of the studied treatment methods made it possible to almost completely prevent the appearance of longitudinal striation, which is characteristic

of spears of the Prius variety. At the same time, no deviations in odor were detected in any treatment, and it remained typical of fresh asparagus.

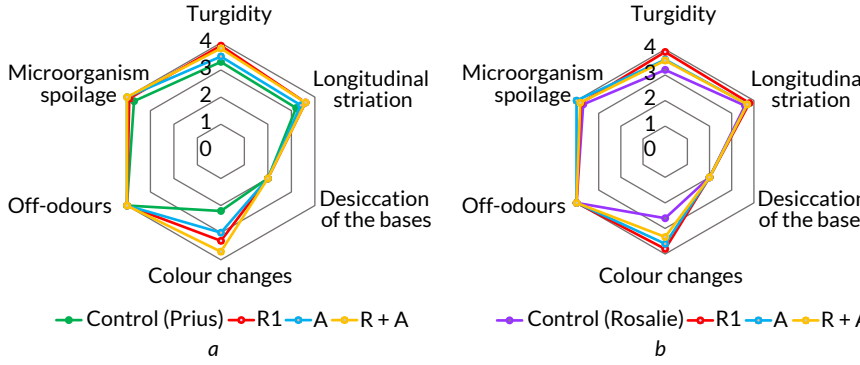

Fig. 10.9 Organoleptic evaluation of asparagus treated with a biopolymer coating based on alginate and rutin at the end of storage: *a* – Prius; *b* – Rosalie

The use of combined biopolymer edible coatings ensured effective preservation of spear turgidity even under extended storage conditions. In addition, such coatings contributed to the stabilization of color, demonstrating a positive effect similar to that of rutin, which confirms the feasibility of their use for maintaining the marketable quality of the product.

One of the key approaches to minimizing losses during the storage of fruit and vegetable products is the reduction of natural weight loss by slowing down respiration and transpiration processes. Transpiration occurs due to the gradient of water vapor partial pressure between the shoot tissues and the surrounding environment. Asparagus is characterized by a high rate of moisture evaporation, which determines its increased sensitivity to dehydration.

The gas exchange of asparagus spears at the beginning of storage, as well as the subsequent dynamics of respiratory processes, shows pronounced variety-specific characteristics. According to the literature, the trends may vary depending on the variety; however, most researchers report a similar pattern of changes at the initial stages of storage. In particular, immediately after cooling, a decrease in respiration intensity is usually observed as a response to the reduction in temperature. After several days, respiration intensity may increase, followed by a subsequent decline. Some studies also describe patterns characterized by a gradual increase in respiratory activity during storage, which highlights the complex and multifactorial nature of the postharvest metabolism of asparagus [24].

According to our results, green asparagus of the Prius variety exhibited a higher respiration rate throughout the entire storage period. Our data regarding the treatment of asparagus spears with sodium alginate-based coatings confirm their effect in reducing the intensity of respiratory metabolism compared with the control samples (**Table 10.4**).

Table 10.4 Respiration rate ($mgCO_2 \times kg^{-1} \times h^{-1}$) of asparagus treated with coatings based on sodium alginate and rutin

Day of storage	Rosalie					Prius				
	Control	R1	A	A + R	LSD_{05}	Control	R1	A	A + R	LSD_{05}
0	106.2	106.2	106.2	106.2	–	94.9	94.9	94.9	94.9	–
1	65.2	72.7	57.0	86.2	5.88	55.4	66.8	55.4	88.7	5.54
7	94.6	82.5	69.7	105.6	7.97	81.4	88.3	91.9	76.9	6.62
12	98.2	113.7	88.4	98.4	8.79	94.5	102.1	75.3	117.3	9.38
21	117.7	87.9	82.1	85.3	10.78	128.4	112.1	67.9	106.6	11.73
37	–	–	–	–		80.0	79.8	115.0	132.0	–

Applying a film coating, which partially isolates the stomata, limits transpiration and creates a barrier to gas exchange, naturally reduces mass loss. Thus, the use of a 1% sodium alginate solution reduced mass loss on the 14th day of storage by 1.8 times for the Rosalie cultivar and by 1.5 times for the Prius cultivar compared with the control. Even higher efficiency was observed for the combined coating based on sodium alginate with the addition of rutin (A+R): mass losses were reduced by 1.9 times for the Prius cultivar and by 2.2 times for the Rosalie cultivar.

The reduction in mass losses in the treated samples was positively reflected in an increase in the yield of marketable produce after storage. Thus, the use of coatings based on sodium alginate and rutin contributes to the extension of the storage life of asparagus spears and the maintenance of their quality attributes.

10.8 Conclusions

It was established that the use of a biopolymer coating based on 1% sodium alginate is an effective method for preserving the quality of asparagus spears of the Prius and Rosalie variety during refrigerated storage. The formation of a semipermeable film on the surface of the spears contributes to a reduction in the intensity of respiration and transpiration, resulting in a decrease in natural mass losses by an average of 1.5 times compared with the control and an increase in the yield of marketable produce.

Adding glycerol at a concentration of 1% to the coating composition did not significantly affect the yield of marketable produce or the reduction of respiratory metabolism.

Treatment of spears with a 1% rutin solution extended the storage duration, reduced the rate of pigment degradation, and helped maintain turgidity and tissue firmness. It was established that a 1% concentration is sufficient to achieve the maximum technological effect without deterioration of organoleptic characteristics.

The most pronounced effect was obtained when using a combined coating (1% sodium alginate + 1% rutin), which combines the barrier properties of the biopolymer with the antioxidant activity of rutin. The use of this composition allowed the shelf life of asparagus spears of both cultivars to be extended by 7 days compared with the control, while minimizing weight loss and ensuring a consistently high yield of marketable produce.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper.

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Data availability

Manuscript has no associated data.

Use of artificial intelligence statement

The authors used the AI assistant Perplexity (Grok 4.1, Perplexity AI) for translation and literature source selection. The authors bear full responsibility for the final manuscript. Generative AI tools are not credited and are not responsible for the final results.

Authors' contributions

Olesia Priss: Supervision, Conceptualization, Methodology, Writing – original draft, Investigation, Project administration.

Liudmyla Kiurcheva: Methodology, Writing – original draft, Writing – review & editing, Investigation.

Pavlo Bulhakov: Methodology, Writing – original draft, Investigation, Visualization, Validation.

Iryna Taraymovich: Writing – original draft, Writing – review & editing, Visualization, Formal analysis, Validation.

Larysa Chepurda: Writing – original draft, Writing – review & editing, Formal analysis.

Sergii Stepanenko: Writing – original draft, Resources, Formal analysis, Visualization.

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CHAPTER 11

Fatty acid composition of total lipids of liver and thigh muscle broiler chickens under the influence of separate and complex action of vitamins E and C

Ludmila Romanovich
Bohdan Kurtyak
Olena Danchenko

Abstract

The section presents the research results devoted to studying the effect of separate and complex inclusion of vitamins E and C in the diet of broiler chickens on the fatty acid composition of total lipids of their liver and skeletal muscles at 41 days of age. Four groups of broiler chickens were formed for the experiment. The control group received standard compound feed (SC); the first experimental group of chickens received vitamin E in addition to SC; the second experimental group received vitamin C; the third experimental group received vitamins E and C simultaneously. The results of the study proved significant differences in the fatty acid composition of total lipids of the liver and thigh muscles of 41-day-old broilers under the influence of separate and complex effects of vitamins E and C. The addition of vitamin E caused an increase in the content of individual saturated fatty acids and a moderate increase in the ω-3 PUFA level in liver tissues. The addition of vitamin C to the chicken diet contributed to an even more pronounced increase in saturated fatty acids, but at the same time a significant decrease in the total PUFA level, especially ω-6. Instead, the combined effect of vitamins E and C led to the most pronounced changes – a significant increase in the total PUFA level (44.06%), a sharp increase in ω-6 (up to 38.3%) and at the same time a decrease in ω-3, which was accompanied by an increase in the ω-6/ω-3 ratio (up to 6.65). In the muscle tissues of chickens receiving vitamin E (groups I and III), a faster increase in the ω-3 PUFA content compared to ω-6 was found against the background of a decrease in the SFA content and MUFA, which contributed both to providing these tissues with a set of necessary PUFA and to a significant increase in the biological value of meat due to optimization of the ω-6/ω-3 PUFA ratio. In chickens to which vitamin C was added, an increase in the antioxidant activity of the tissues

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was accompanied by an increase in the total SFA content, and the ω-6/ω-3 PUFA ratio remained at the level of the control group (35.84).

Keywords

Broiler chickens, liver, lipid peroxidation, fatty acid composition, vitamins E and C, ω-6 and ω-3 polyunsaturated fatty acids.

11.1 The problem of antioxidant protection of broiler chickens and the role of vitamins E and C in its provision

Poultry meat remains the main type of meat product on the domestic Ukrainian market. Poultry farming provides a significant share of the population's needs for balanced animal proteins. At the same time, even in wartime, the export of poultry meat, primarily chicken, is an essential activity of the Ukrainian agriculture, as Ukraine occupies one of the leading positions among the main exporters of this product [1]. Poultry meat is characterized by high nutritional value, digestibility and relative affordability, which determines stable demand for this product [2]. The full-scale invasion of the Russian Federation in 2022 radically changed the landscape of Ukrainian poultry farming. In addition to the direct destruction of capacity, the industry faced many other problems associated with disruption of logistics processes, energy instability and limited refrigeration infrastructure capabilities. However, despite many negative factors, the level of poultry production during the 4 years of the war decreased by only 7.2% [3] and now the efforts of scientists and specialists in the agricultural sector are aimed at the restoration of poultry farming and its further development.

Industrial crosses of broilers are characterized by a high level of metabolic processes, which is accompanied by intensification of lipid peroxidation (LPO) in poultry tissues and the accumulation of reactive oxygen species (ROS) [4]. These changes are more pronounced during critical periods of poultry ontogenesis and are caused by the influx of stress factors (vaccination) and intensive growth [5, 6]. The processes of raising poultry are associated with a number of stress factors, from hatching to slaughter. Excessive formation of reactive oxygen species and oxidative stress are the main negative factors that cause most of the losses of poultry. Therefore, the development of a system of optimal antioxidant supplements to maintain effective antioxidant protection and redox balance in the poultry body is an urgent task.

Polyunsaturated fatty acids (PUFA) of the ω-6 (linoleic, arachidonic, eicosatrienoic) and ω-3 (linolenic, eicosapentaenoic, docosahexaenoic) families play a key role and occupy a prominent place in the structure of cell membranes and the regulation of

metabolic processes. The high PUFA content in phospholipids improves the fluidity and functionality of cell membranes, which is important for the normal functioning of poultry organs and systems. On the other hand, the content and ratio of ω-6 and ω-3 PUFA in muscles and liver significantly affect the nutritional value and quality of poultry products. Increasing the proportion of ω-3 PUFA in meat lipids increases its biological value for humans, as these acids have antiatherogenic, cardioprotective and anti-inflammatory properties. It has been proven that ω-3 PUFA are important modulators of immune function and the nervous system, and a high ω-6 PUFA content without sufficient ω-3 intake is associated with an increased risk of metabolic and inflammatory disorders [7, 8]. A low ω-6/ω-3 ratio (close to 4:1) is considered optimal for human nutrition, while an excess of linoleic acid against the background of a deficiency of ω-3 PUFA can cause undesirable health consequences [9]. In birds, an increase in the ω-3 PUFA content in tissues also has a positive effect: there is evidence that moderate enrichment of the diet with ω-3 PUFA enhances antioxidant status (glutathione peroxidase activity) and reduces the level of lipoperoxidation in chickens. In addition to their antioxidant and anti-inflammatory roles, ω-3 fatty acids are thought to regulate platelet homeostasis and reduce the risk of thrombosis. Conversely, the presence of a large amount of ω-6 PUFA in tissue lipids with insufficient levels of antioxidants leads to intensive fat oxidation and accumulation of lipid peroxidation products, which leads to a deterioration in the quality of the resulting meat [10, 11]. Therefore, in modern poultry farming, considerable attention is paid to optimizing the fatty acid composition (FAC) of the broiler diet – in particular, adding sources of ω-3 PUFA (linseed oil or fish oil) – in combination with appropriate antioxidant provision. This allows for a significant reduction in the ω-6/ω-3 ratio in poultry fats and obtaining meat enriched with beneficial ω-3 acids and capable of contributing to the prevention of cardiovascular and metabolic diseases in humans [7].

It is known that the most powerful natural antioxidants, vitamins E and C, perform complementary functions in the poultry body. Fat-soluble vitamin E (tocopherol) is integrated into the phospholipid bilayer of membranes and protects PUFA from peroxidation by uncoupling free radical chains. Due to this, tocopherol stabilizes cellular and subcellular membranes (in particular mitochondria), prevents oxidative damage to lipids and proteins, and supports the functional activity of various systems of the broiler body during stress [12, 13]. In addition to its direct antioxidant action, tocopherol also performs a number of other physiological functions. Modern studies show that vitamin E is an important regulator of cellular processes, an immunomodulator, an anti-inflammatory factor, and a neuroprotector, therefore it is a factor in the general adaptive resistance of the poultry body in conditions of intensive production [14, 15].

At the same time, vitamin C (ascorbic acid) is a water-soluble antioxidant that is synthesized in the body of chickens, but its reserves are quickly depleted under the influence of stress and high metabolic rate. In such cases, additional administration of ascorbic acid (200–250 mg/kg of feed) helps to maintain the normal course of metabolic processes, increase the productivity and quality of broiler meat by enhancing their antioxidant and immune potential. It is important that vitamin C restores the activity of vitamin E, regenerating its active form from the tocopherol radical and thereby prolonging the antioxidant effect of tocopherol in tissues [16, 17]. Many studies on broilers confirm that the complex use of vitamins E and C, as well as trace elements (Selenium, Zinc) provides better protection against oxidative stress than the use of each of these substances separately [18]. In particular, combined supplementation of the diet with high doses of vitamin E and coherent antioxidants significantly reduces the in vivo concentration of lipid peroxidation products and improves the preservation and quality of broiler meat during storage. Antioxidant vitamins also indirectly affect lipid metabolism in poultry. It has been established that increasing the tocopherol level in the diet of broilers contributes to the normalization of lipid profile indicators – reducing the content of cholesterol and triacylglycerols in meat, and can also modulate the relative content of individual fatty acids in tissues. According to R. Voloshyn et al. [19], an increase in the concentration of vitamin E in the feed of broilers by 4–16 times compared to the norm caused a dose-dependent increase in the proportion of arachidonic acid (ω-6 PUFA) in liver lipids (by 1.3–1.5 times compared to the control) with a simultaneous decrease in the level of stearic acid, which indicates stimulation of the biosynthesis of arachidonic acid by tocopherol.

Our previous studies [20, 21] confirmed that the level of lipid hydroperoxides and end products of lipoperoxidation in the blood plasma of broilers sharply increases during increased growth processes in poultry and in the period after vaccination. Excessive accumulation of LPO products is one of the factors of damage to cell membranes and deterioration of both poultry productivity and meat quality after slaughter. Additional introduction of natural antioxidants into the poultry diet can restrain these negative processes. It has been established that increased doses of vitamins E and C in the diet of broilers cause a decrease in the content of intermediate and final products of lipid peroxidation in their tissues and enhance the antioxidant defense of the bird's body, especially under conditions of stress factors. It has been proven [22] that increased levels of tocopherol (0.1 g/kg) and ascorbic acid (0.25 g/kg) in the diet of broiler chickens significantly reduce the accumulation of lipid hydroperoxides and TBA-active products in the blood of 41-day-old broilers, and the lowest levels of lipid peroxidation were observed in chickens that received vitamin E and C supplements at the same time. This indicates the effectiveness of antioxidant

prevention of oxidative stress in fast-growing broilers and the possible synergistic effect of the antioxidant effect of vitamins E and C under the action of stress factors. At the same time, questions regarding the influence of these vitamins on the fatty acid spectrum of lipids in broilers remain unclear. Therefore, the aim of our research was to determine the effect of individual and combined effects of vitamins E and C on the fatty acid composition of total lipids in liver and skeletal muscle in 41-day-old broiler chickens.

11.2 Materials and methods of research

Experimental studies were conducted in a farm in Zolochiv district, Lviv region, on broiler chickens of the Ross-308 cross from 1 to 41 days of age, kept on the floor on deep litter, with free access to feed and water. Technological parameters of broiler farming met all zootechnical requirements. The experiment was conducted on 4 groups of broiler chickens of 100 heads each. The control group was fed standard compound feed (SC) in accordance with the existing standards recommended for the ROSS-308 cross. The first experimental group of chickens, in addition to SC, received vitamin E 100 mg/1 kg of compound feed. The second experimental group received vitamin C 250 mg/1 kg of compound feed. The third experimental group received vitamin C 250 mg/1 kg and vitamin E 100 mg/1 kg of compound feed in addition to the diet. The composition and nutritional value of compound feed for broiler chickens is given in **Table 11.1**.

Chickens were vaccinated according to the preventive vaccination schedule on the farm: against infectious bronchitis at 11 days of age; against Newcastle disease at 13 days of age; against infectious bursal disease at 15 days of age. After slaughtering chickens at 41 days of age, liver and thigh muscle samples were taken for biochemical studies. Before slaughter, broilers were kept for 6 hours without feed and 3 hours without water. Liver and muscle samples were frozen and stored in liquid nitrogen, then ground into a powder, which was used for further studies. The fatty acid composition (FAC) of total lipids was determined in the liver and muscle according to DSTU ISO 5508-2001 "Animal and vegetable fats and oils". Sample preparation was carried out according to the method of DSTU ISO 5509-2002 "Animal and vegetable fats and oils". Chromatographic analysis of pre-methylated fatty acids was performed on a Trace Ultra gas chromatograph with a flame ionization detector, on a highly polar capillary column SP-2560 (Supelco). The method limit is 0.01%. Identification of fatty acids is carried out using the analytical mixture of fatty acids SupelcoTM 37 Compone FAME MIX, 100 mg Nea [22].

Table 11.1 Composition and nutritional value of compound feed

100 grams of compound feed contains, %				
Metabolic energy, Kcal	303.41	314.46	323.33	328.11
Crude protein	22.20	20.21	19.00	18.00
Crude fat	6.28	8.85	9.78	10.14
Crude fat extracted	5.55	8.08	9.02	9.39
Crude fiber	3.19	4.09	4.19	4.17
Crude ash	6.10	5.11	4.51	4.29
Moisture	11.44	9.75	9.98	10.18
Lysine	1.38	1.28	1.16	1.07
Methionine	0.63	0.60	0.54	0.50
Methionine + cystine	0.99	0.93	0.85	0.80
Threonine	0.88	0.84	0.77	0.72
Tryptophan	0.27	0.24	0.22	0.21
Isoleucine	0.94	0.85	0.79	0.74
Arginine	1.46	1.33	1.26	1.19
Valine	1.06	1.00	0.91	0.85
Chlorine	0.25	0.26	0.25	0.25
Potassium	0.98	1.07	0.99	0.93
Sodium	0.14	0.14	0.14	0.14

The obtained digital data were statistically processed using the computer program "Microsoft Excel". The degree of probability of comparative data was assessed by the Student's test (t). The difference was considered significant at ($p < 0.05–0.001$).

Studies on broiler chickens were carried out in compliance with the provisions of the Council of Europe Convention of (04.08.1997) and the resolution of the Cabinet of Ministers of Ukraine of 24.08.2002, No. 1256.

11.3 Results of the studies and their discussion

Comparative analysis of the fatty acids of liver lipids of 41-day-old broiler chickens of the control and I experimental groups shows that under the action of vitamin E supplements (**Table 11.2**) there was a redistribution of fatty acids in the direction of increasing the content of saturated fatty acids by 12.6%, mainly due to an increase in the content of palmitic acid (by 25.4%, $p \leq 0.01$) with a simultaneous decrease in the total PUFA content by 11.0%. At the same time, the MUFA content in chickens of the I experimental group remained at a stable level. Within PUFA, a decrease in the

ω-6 PUFA content by 25.4% was established, which occurred mainly due to a decrease in the content of essential linoleic acid (18:2, by 37.6%). At the same time, the content of longer-chain ω-6 arachidonic acid (20:4) increased by 33.5%. Under the action of vitamin E, against the background of a decrease in the ω-6 PUFA content, an increase in the ω-3 PUFA content by 45.1% was established, including linolenic (18:3) by 15.2% ($p \leq 0.01$), docosapentaenoic (22:5) by 2.19 times, docosahexaenoic (22:6) by 33.6%. Such changes in FAC, caused by an increase in the content of vitamin E in the diet of chickens of the I experimental group, were accompanied by a significant decrease in the ratio of ω-6/ω-3 PUFA compared to this indicator in broilers of the control group (by 1.94 times), (**Fig. 11.1**), which is evidence of an increase in the FAC biological value of liver lipids under the action of vitamin E.

Table 11.2 Fatty acid composition of liver lipids of 41-day-old broiler chickens under the influence of vitamin E and C supplements to the diet, % ($M \pm m$; $n = 3$)

Fatty acid	Groups of broiler chickens			
	C	I E	II E	III E
C14:0	0.18 ± 0.006	0.21 ± 0.006*	0.30 ± 0.006***	0.19 ± 0.006
C16:0	17.18 ± 0.038	21.5 ± 0.121***	20.80 ± 0.344***	19.78 ± 0.288***
C16:1 ω-9	0.40 ± 0.008	0.42 ± 0.012	0.31 ± 0.006**	0.31 ± 0.006**
C17:0	0.047 ± 0.003	0.053 ± 0.003	0.047 ± 0.003	0.016 ± 0.003*8
C18:0	14.4 ± 0.130	14.20 ± 0.026	19.51 ± 0.155***	9.35 ± 0.112***
C18:1 ω-9	29.64 ± 0.061	29.77 ± 0.44	30.77 ± 0.12**	25.22 ± 0.049***
C18:2 ω-6	24.58 ± 0.035	15.33 ± 0.072***	13.5 ± 0.032***	30.47 ± 0.348***
C20:0	0.39 ± 0.012	0.31 ± 0.006**	0.47 ± 0.012**	0.50 ± 0.006**
C20:1 ω-9	0.39 ± 0.006	0.43 ± 0.007*	0.48 ± 0.006***	0.57 ± 0.009***
C18:3 ω-3	2.83 ± 0.021	3.26 ± 0.006***	2.33 ± 0.009***	2.49 ± 0.012***
C20:3 ω-6	1.20 ± 0.008	1.96 ± 0.012***	1.92 ± 0.015***	2.81 ± 0.017***
C20:4 ω-6	3.28 ± 0.035	4.38 ± 0.038***	3.59 ± 0.018**	4.17 ± 0.031***
C22:2 ω-6	0.68 ± 0.012	0.52 ± 0.012***	0.85 ± 0.015***	0.85 ± 0.0015***
C20:5 ω-3	0.77 ± 0.006	1.69 ± 0.036***	1.14 ± 0.009***	0.76 ± 0.009
C22:5 ω-3	0.90 ± 0.012	1.94 ± 0.111***	0.94 ± 0.012	0.65 ± 0.009***
C22:6 ω-3	3.15 ± 0.023	4.21 ± 0.012***	3.14 ± 0.018	1.86 ± 0.014***
SFA	32.2	36.27	41.12	29.83
MUFA	30.43	30.62	31.56	21.10
PUFA	37.39	33.29	27.41	44.06

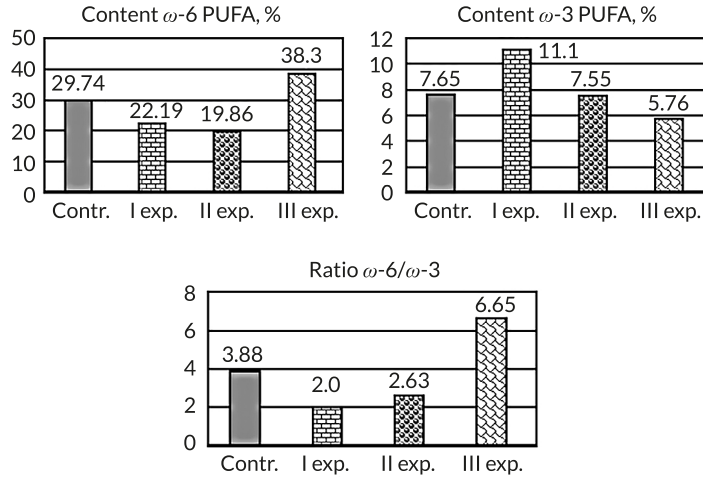

Fig. 11.1 Changes in the content of ω-6 and ω-3 PUFAs and their ratio in the composition of total lipids in the liver of broiler chickens of the experimental groups compared to the control

Comparative analysis of the fatty acid composition of liver lipids of broilers of the control and II experimental groups shows that under the action of ascorbic acid supplements, the total level of saturated fatty acids increased by 27.7% with a simultaneous decrease in the total PUFA content by 26.7%, mainly due to ω-6 PUFA, the content of which in the liver of broilers of the II experimental group was 33.2% lower than the corresponding indicator in the control group. At the same time, the content of essential ω-6 linoleic (18:2) acid in the liver of broilers under the action of vitamin C decreased by 45.1%. Such a significant decrease in the ω-6 PUFA content against the background of a stable ω-3 PUFA level contributed to a decrease in the ratio of ω-6/ω-3 PUFA by 32.2% compared to the corresponding indicator in broilers of the control group.

A comparative assessment of the effect of vitamins E and C on the directions of the main changes in FAC in the liver of broilers of groups I and II shows that in both groups of broilers the increase in the antioxidant status of the tissues [23] occurred against the background of an increase in the content of saturated FA with a stable MUFA level. At the same time, the increase in the level of saturated fatty acids in chickens of groups I and II of the study occurred with a simultaneous increase ($p < 0.001$) in the content of palmitic (16:0), and in broilers of group II of the study also stearic (18:0) fatty acids. Regarding changes in the PUFA content, in both experimental groups (I and II) the decrease in ω-6 PUFAs was mainly due to a decrease in the content of linoleic ($p < 0.001$) acid, respectively by 37.6 and

45.1% against the background of a simultaneous increase in the content of longer-chain ω-6 arachidonic acid (by 33.5% ($p < 0.001$) and 9.5% ($p < 0.01$) respectively). At the same time, a significant increase in the content of all identified ω-3 PUFA was found in the liver of chickens of experimental group I, and in the liver of chickens of experimental group II the content of ω-3 acids generally remained at a stable level (linolenic acid even significantly decreased).

The main differences in the changes in the lipid composition of the liver of chickens of the I and II experimental groups are that under the influence of vitamin E supplementation, oppositely directed changes in the ω-6 PUFA content (decrease) and ω-3 PUFA (increase) occurred, which contributed to a decrease in the ratio of ω-6/ω-3 PUFA in the liver of broilers of the I experimental group to the lowest value in this experiment. In chickens of the II experimental group, the addition of vitamin C to the diet did not cause significant changes in the ω-3 PUFA content, but a decrease in the ω-6 PUFA content contributed to an improvement in the ratio of ω-6/ω-3 PUFA.

Thus, the conducted studies have shown that the addition of vitamin C to the diet of chickens has a lesser effect on the fatty acid profile in the liver, but its presence is necessary to maintain the pro-oxidant-antioxidant balance in the bird's body, especially under the influence of stress factors.

When studying the total lipid composition of liver tissues of chickens of III experimental group, which received a complex supplement of vitamins E and C, changes were recorded in a different direction than under the separate action of these vitamins. In particular, in the liver tissues of chickens of the 3rd experimental group, the level of saturated fatty acids and MUFA decreased by 7.4 and 30.7%, respectively, mainly due to a decrease in the content of stearic (18:0) and oleic (18:1) acids. At the same time, against the background of an increase in the total PUFA content (by 17.8%), an increase in the content of all ω-6 PUFA by 28.8% was established, including linoleic (18:2) by 24.0% ($p \leq 0.001$) and arachidonic (20:4) by 27.1% ($p \leq 0.001$). At the same time, in the liver of broilers of this experimental group, a decrease in the total ω-3 PUFA level by 24.7% was found due to a significant decrease in the content of linolenic (18:3), docosapentaenoic (22:5) and docosahexaenoic (22:6) fatty acids, which led to an increase in the ratio of ω-6/ω-3 PUFA by 71.4%.

The nutritional value of broiler chicken meat is largely determined by the fatty acid composition of total lipids, in particular the content and quality of PUFA. Analysis of the fatty acid composition of broiler chicken thigh muscles of the control group (**Table 11.3**) shows that 65.6% of the mass of all fatty acids are SFA and MUFA. Among PUFA (33.66%), only 0.93% is ω-3PUFA, and their main part is linoleic ω-6 acid. Accordingly, in terms of the ω-6/ω-3 PUFA ratio, the thigh muscles of chickens in the control group do not meet the recommendations of scientists and require

correction of the FAC by additional enrichment of their lipid component with ω-3 PUFA (**Fig. 11.2**).

Increasing the level of vitamin E in the diet of broiler chickens of experimental group I caused a decrease in the relative level of SFA and MUFA, respectively by 17.5 and 8.2% and an increase in the relative PUFA content in the composition of thigh muscle lipids by 22.4%. The decrease in the SFA level in the composition of thigh muscle lipids of chickens of this group occurred mainly due to a decrease ($P < 0.001$) in the content of palmitic (16:0) and stearic (18:0) fatty acids, and MUFA – a decrease in the level of oleic (18:1) acid.

Table 11.3 Fatty acid composition of total lipids of thigh muscles of 41-day-old broiler chickens under the influence of dietary supplements of vitamins E and C, % ($M \pm m$; $n = 3$)

Fatty acid	Groups of broiler chickens			
	C	I E	II E	III E
(8:0)	0.07 ± 0.006	0.05 ± 0.006	0.07 ± 0.006	0.04 ± 0.003*
(10:0)	0.11 ± 0.006	0.37 ± 0.265	0.13 ± 0.006	0.10 ± 0.006
(12:0)	0.26 ± 0.005	0.23 ± 0.006*	0.21 ± 0.006**	0.2 ± 0.006**
(14:0)	0.63 ± 0.012	0.49 ± 0.0012**	0.58 ± 0.003*	0.52 ± 0.008**
(15:0)	0.2 ± 0.006	0.17 ± 0.006*	0.22 ± 0.006	0.19 ± 0.006
(16:0)	21.32 ± 0.187	17.13 ± 0.052***	23.16 ± 0.056***	18.54 ± 0.055***
(16:1)	2.69 ± 0.015	1.9 ± 0.012***	3.16 ± 0.032***	2.20 ± 0.012**
(17:0)	0.12 ± 0.003	0.1 ± 0.006	0.11 ± 0.003	0.1 ± 0.006
(18:0)	11.26 ± 0.08	9.48 ± 0.180***	10.35 ± 0.027***	8.36 ± 0.132***
(18:1)	28.3 ± 0.29	26.53 ± 0.14**	25.25 ± 0.10***	25.87 ± 0.02***
(18:2) ω-6	30.44 ± 0.30	38.00 ± 0.295***	32.14 ± 0.055**	38.33 ± 0.054***
(20:0)	0.11 ± 0.007	0.09 ± 0.006	1.17 ± 0.009**	0.13 ± 0.009
(18:3) ω-3	0.10 ± 0.008	2.18 ± 0.036***	0.19 ± 0.006**	1.43 ± 0.045***
(20:1)	0.3 ± 0.012	0.34 ± 0.006*	0.20 ± 0.003**	0.50 ± 0.015***
(20:3) ω-6	0.33 ± 0.009	0.31 ± 0.006	0.41 ± 0.006**	0.41 ± 0.006**
(20:4) ω-6	1.96 ± 0.054	1.54 ± 0.072**	2.57 ± 0.034***	1.54 ± 0.072**
(20:5) ω-3	0.16 ± 0.006	0.2 ± 0.006**	0.12 ± 0.006**	0.23 ± 0.009**
(24:1)	0.26 ± 0.006	0.20 ± 0.009**	0.30 ± 0.009*	0.26 ± 0.009
(22:5) ω-3	0.21 ± 0.009	0.30 ± 0.009***	0.26 ± 0.006*	0.34 ± 0.006***
(22:6) ω-3	0.46 ± 0.009	0.66 ± 0.009***	0.41 ± 0.006*	0.71 ± 0.006***
SFA	34.08	28.11	36.0	28.18
MUFA	31.55	28.97	28.91	28.83
PUFA	33.66	41.19	34.1	42.99

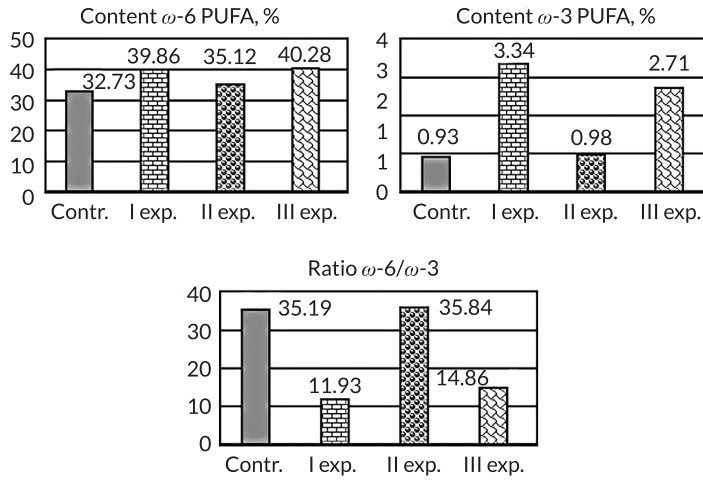

Fig. 11.2 Changes in the content of ω-6 and ω-3 PUFA and their ratio in the composition of total lipids in the thigh muscles of broiler chickens of the experimental groups compared to the control

Additional enrichment of the diet of chickens with vitamin E caused a significant increase in the PUFA level in the composition of thigh muscle lipids. Against the background of an increase in the total ω-6 PUFA content by 21.8%, including the content of linoleic acid by 24.8% ($p < 0.001$), the total ω-3 PUFA content increased by 3.5 times. At the same time, a higher ($p < 0.01–0.001$) content of linolenic (18:3), eicosapentaenoic (20:5), docosapentaenoic (22:5) and docosahexaenoic (22:6) fatty acids was recorded in the lipid composition of the thigh muscles of chickens of this group compared to the control group. Such a PUFA redistribution under the influence of vitamin E contributed to a significant reduction in ω-6/ω-3 PUFA and an approach of this indicator to the recommended level [24].

The increase in vitamin C in the diet of chickens of the experimental group II contributed to certain fluctuations in the content of individual acids, in particular, a probable increase in the content of linoleic (18:2) and arachidonic (20:4) fatty acids and a decrease in the level of oleic (18:1) acid in the composition of thigh muscle lipids. However, these changes did not significantly affect the total SFA content, MUFA and PUFA and the ratio of ω-6/ω-3 PUFA in the composition of muscles, which indicates a significant deficiency of ω-3 PUFA in these tissues.

With the combined addition of vitamins E and C to the diet of broiler chickens, the changes in the SFA content, MUFA and PUFA observed were similar to those in

the experimental group I (application of vitamin E). In particular, in the composition of thigh muscle lipids of chickens of the experimental group III, a lower SFA content, MUFA and a higher PUFA content were recorded compared to the control.

The decrease in the relative level of SFA and MUFA in the composition of the muscles of chickens of this group was also similar to the experimental group I and occurred mainly with a simultaneous significant decrease in the content of palmitic (16:0), stearic (18:0) and oleic (18:1) fatty acids.

The PUFA content in the composition of the thigh muscles of chickens of the experimental group III was 9.3% higher than in the control group. The increase in the level of polyunsaturated fatty acids in the composition of lipids of the thigh muscles of chickens of this group was mainly due to an increase in the content of linoleic acid. Thus, the content of linoleic acid in the composition of lipids of the thigh muscles of chickens of the experimental group III was 7.9% ($p < 0.001$) higher than in the control group.

The increase in the level of vitamin C in the diet of chickens of the experimental group II contributed to certain fluctuations in the content of individual acids, in particular, a probable increase in the content of linoleic (18:2) and arachidonic (20:4) fatty acids and a decrease in the level of oleic (18:1) acid in the lipid composition of thigh muscles. However, these changes did not significantly affect the total content of SFA, MUFA and PUFA and the ratio of ω-6/ω-3 PUFA in the muscles of chickens of the experimental group II, which indicates a persistent deficiency of ω-3 PUFA in the muscles of chickens of this group. Therefore, taking into account previously published research results [25], the addition of vitamin C to the diet of broilers contributes to the inhibition of lipid peroxidation processes and an increase in the level of ω-6 PUFA. However, according to the ratio of ω-6/ω-3 PUFA in the muscles of chickens of the experimental group II, no increase in biological value was detected compared to the control, which proves the need for additional enrichment of the diet of this group of chickens with ω-3 PUFA.

With the combined addition of vitamins E and C to the diet of broiler chickens (experimental group III), changes in the SFA content, MUFA and PUFA observed in their thigh muscles were similar to those in experimental group I. The decrease in SFA, MUFA in the muscles of chickens of this group occurred mainly with a simultaneous significant decrease in the content of palmitic (16:0), stearic (18:0) and oleic (18:1) fatty acids. The PUFA content in the thigh muscles of chickens of experimental group III was 27.7% higher than in the control. At the same time, an increase in the ω-6 PUFA content in the muscles of chickens of this group by 23.1% was established (mainly due to the content of linoleic acid (by 25.9%, $p < 0.001$). At the same time, the ω-3 PUFA content in the muscles of chickens of the III experimental group

exceeded the corresponding indicator of the control by 2.91 times. The content of all ω-3 PUFA (linolenic, eicosapentaenoic, docosapentaenoic and docosahexaenoic acids) was higher ($p < 0.01–0.001$), than in the lipids of the thigh muscles of broilers of the control group. These data indicate a stimulating effect of vitamin E supplementation to the diet of chickens separately, as well as in combination with vitamin C, on the PUFA content in the lipids of the thigh muscles of chickens. The increase in the PUFA content, and especially linoleic and linolenic fatty acids in the lipids of the thigh muscles of chickens can be explained by the stimulating effect of tocopherol on the activity of enzyme systems involved in the synthesis of these fatty acids [26]. In addition, the positive side of the effect of vitamin E and C supplementation to the diet is the increase in the nutritional value of broiler chicken meat due to a decrease in the ratio of ω-6/ω-3 PUFA content relative to the control.

In general, the results of the conducted studies of the FAS of total lipids of liver tissues of 41-day-old broiler chickens indicate different changes in the content of individual fatty acids with separate and combined use of vitamin E and C supplements. The data on the PUFA content in the composition of total lipids of liver tissues of broiler chickens of the experimental groups deserve special attention. In particular, under the conditions of separate use of vitamin E and C supplements to the feed of broiler chickens, a significant decrease in the relative content of linoleic acid in the composition of total lipids of liver tissues was recorded. At the same time, the decrease in the content of linoleic acid in the liver of chickens of the experimental groups I and II during the specified period of research was accompanied by a simultaneous increase in the relative proportion of arachidonic acid. Linoleic acid is not synthesized in the poultry body, on the other hand, it is a precursor of arachidonic acid [4]. Thus, it is logical to assume that the decrease in the content of linoleic acid may also be associated with its use in the synthesis of longer-chain unsaturated arachidonic acid under the influence of the studied vitamin supplements. It was found that vitamin E increased the content of arachidonic acid (20:4 *n*-6) in the liver to a greater extent and simultaneously reduced the level of its precursor linoleic acid (18:2 *n*-6) – probably due to the activation of the enzymatic conversion of ω-6 PUFA to long-chain derivatives. On the other hand, in chickens of experimental group I, an increase in the ω-3 PUFA level (linolenic, eicosapentaenoic, docosapentaenoic and docosahexaenoic acids) was observed, which is consistent with the results of studies [24], the authors of which noted that the addition of 200 mg/kg of vitamin E led to an increase in the ω-3 PUFA content and a decrease in the ω-6/ω-3 ratio in broiler meat. According to the researchers, tocopherol selectively protects the most unsaturated fatty acid molecules from oxidation, due to which more long-chain ω-3 PUFA (EPA, DHA) accumulate in the lipids of the muscle tissue of broilers

and relatively fewer ω-6 derivatives, which compete with them for metabolic enzymes and inclusion in the phospholipids of cell membranes. At the same time, it is in the liver (as an organ with a high intensity of lipid metabolism) that the effects of vitamin E on the fatty acid composition are probably most pronounced. Vitamin C does not exert a significant effect on the fatty acid profile in the tissues, but its presence is necessary to maintain the antioxidant status and peroxide balance of the bird's body, especially under the influence of stress factors.

Thus, the results of the conducted studies indicate that feeding broiler chickens with supplements containing vitamins E and C during their intensive growth period contributes to an increase in lipid synthesis in their liver and the deposition of synthesized lipids in skeletal muscles. At the same time, these processes are accompanied by a redistribution of the ratio of individual lipid classes in the organs and tissues of chickens, which was shown in our previous works [20, 21].

The doses of vitamins used were determined based on the results of studies [22–25], which confirmed the dose-dependent effects of vitamin E and C supplements on growth performance, nutrient digestibility, and hematological parameters in broiler chickens.

The results of our studies are consistent with those of [26–28], which showed that increasing the vitamin E level (an additional 200 mg/kg) was accompanied by an increase in the ω-3 PUFA content and a decrease in the ω-6/ω-3 ratio in muscle. The effect of vitamin E supplementation on fatty acid dehydrogenase activity was also studied [29, 30]. However, most researchers believe that tissue FA is primarily determined by diet composition, and that maintenance of the fatty acid profile is ensured by antioxidant vitamins, primarily vitamin E, by reducing lipid peroxidation [31].

11.4 Conclusions

Significant differences in the fatty acid composition of total lipids of the liver and thigh muscles of 41-day-old broilers were established under the influence of separate and complex action of vitamins E and C. In the liver tissues of chickens under the individual influence of vitamins E and C, an increase in the SFA content was established with a simultaneous decrease in PUFA due to ω-6 PUFA. Under the joint action of vitamins E and C, oppositely directed changes in SFA and PUFA were established, which caused an increase in the ratio ω-6/ω-3 by 71.3% (up to 6.65). However, even at this level, this indicator remains within acceptable limits. In the muscle tissues of chickens receiving vitamin E (groups I and III), a more rapid increase in the ω-3 PUFA content compared to ω-6 was found against the background of a decrease in the SFA content and MUFA, which contributed both to providing these tissues

with a set of necessary PUFAs and to increasing the biological value of meat by optimizing the ratio of ω-6/ω-3 PUFA.

Thus, the results of the studies suggest that the effect of vitamins E and C on FA has a certain tissue specificity. While the directions of FA changes in muscle tissue in groups I and II coincide, the different directions of changes in FAC in liver tissue are determined by the intensity of metabolic processes in this organ. Further work by scientists and producers should be aimed at developing diets for poultry and pets rich in omega-3 PUFA, vitamins and phytonutrients, but low in omega-6 PUFA, which will ultimately improve the health and well-being of consumers [28]. In addition, healthy nutrition requires ensuring the required composition of polyunsaturated fatty acids (PUFA), vitamins and microelements in meat throughout the entire processing and storage process – from farm to table. Omega-3 PUFA-rich diets for pets offer numerous economic, environmental, and social benefits for meat consumers.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper, as well as the published research results, including the financial aspects of conducting the research, obtaining and using its results, as well as any non-financial personal relationships.

Financing

The study was performed without financial support.

Data availability

The data that support the findings of this study will be made available by the authors on reasonable request.

Use of artificial intelligence statement

The authors confirm that they did not use artificial intelligence technologies when creating this work

Authors' contributions

Ludmila Romanovich: Conceptualization, Literature review, Conducting experimental studies, Data analysis, Writing – original version.

Bohdan Kurtyak: Conceptualization, Methodology, Organization of the study, Formal analysis, Writing – review and editing.

Olena Danchenko: Theoretical framework, Literature review, Interpretation of results, Visualization, Writing – review and editing.

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CHAPTER 12

Microbiological stability of filled gingerbread: problems and technological solutions

Sergiy Smirnov
Olesia Priss
Svitlana Danylenko

Abstract

Microbial stability remains a major challenge in extending the shelf life of bread and flour-based confectionery products. This issue is particularly relevant for filled gingerbread (pryaniki), where consistent product quality throughout the distribution period is essential for both consumer acceptance and manufacturer reputation. The present paper combines a narrative review of the scientific literature with a practical case study to examine the factors affecting microbial spoilage of filled gingerbread and to discuss technological approaches for improving their microbial stability.

The review summarizes current knowledge on the shelf life of bread and related bakery products, with filled gingerbread considered as a representative example of a multi-component system. In such products, the crumb and filling differ in composition and physicochemical properties, which can lead to internal moisture redistribution during storage. Literature data indicate that shelf-life limitations are associated not only with staling but also with moisture migration and post-baking contamination. Local increases in water activity may occur at the crumb-filling interface, creating microenvironments that favor the growth of xerotolerant molds and osmophilic yeasts. In addition, air and contact surfaces in cooling and packaging areas represent important contamination pathways.

The review is complemented by a case study of filled gingerbread, in which selected physicochemical and microbiological indicators were evaluated during storage. The combined analysis highlights the importance of a hurdle strategy that integrates control of component aw, hygienic zoning after baking, appropriate barrier packaging and headspace management, as well as formulation adjustments and complementary preservation measures.

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Keywords
Filled gingerbread, microbiological spoilage, xerophilic molds, water activity, shelf life, contamination control, bakery products.

12.1 Introduction

Gingerbread represents a significant share of flour confectionery products in Europe and Ukraine. These products have long cultural and gastronomic traditions and are strongly associated with festive baking, most often with Christmas. Gingerbread is a type of cookie distinguished mainly by a high content of spices (cloves, cinnamon, ginger, cardamom, nutmeg, and others).

The recipe, the combination of spices, and technological methods differ depending on regional preferences. In Northern European countries, gingerbread is often made from thin, crispy spiced dough (pepparkakor in Sweden, pepperkaker in Norway, piparkakut in Finland, peberkager in Denmark). Similar thin and crispy products with a pronounced aroma, known as speculaas (speculoos), are produced in the Netherlands and Belgium. Gingerbread products in Central Europe usually have a moderately dense and elastic structure and a characteristic aromatic profile (Polish pierniki, Czech perníky). The well-known Nuremberg gingerbread Lebkuchen is characterized by a minimal amount of flour and a high proportion of nuts. Softer and slightly more airy gingerbread products are traditional for Southern Europe, such as pan de jengibre, as well as polvorones and mantecados in Spain, and panpepato and panforte in Italy. These historically formed consumption traditions support stable demand for gingerbread and contribute to the development of modern production technologies.

Despite significant differences in formulation and processing technologies, gingerbread products share a common characteristic: an extended shelf life. This is mainly due to the antibacterial properties of spices [1, 2]. In general, spicy-aromatic components are added to the formulation of bakery and confectionery products to provide specific organoleptic characteristics, but they may also contribute to extending the shelf life [3, 4].

Unlike most flour confectionery products with a short sales period, gingerbread is considered a relatively stable product. However, even at relatively low values of water activity (a_w) and high osmotic concentration (sugars, invert syrups, honey components), it remains vulnerable to the development of xerotolerant and xerophilic mycobiota [5].

An important stage in the development of gingerbread technology and assortment was the use of marzipan, nut, and fruit-berry fillings. This made it possible to

obtain new flavor-aroma profiles and texture characteristics of the products. At the same time, products with fillings have a higher risk of microbiological spoilage. This risk increases due to the non-uniform moisture profile in different parts of the product, especially at the phase boundary between crumb and filling. The filling often releases free moisture and locally increases aw in the contact zone with the baked layer, creating conditions for the growth of microorganisms.

Therefore, the aim of this work was to discuss the causes of microbiological spoilage in filled gingerbread and to identify possible technological solutions.

12.2 Factors affecting the microbiological stability of filled gingerbread

The main causes of spoilage in flour-based confectionery products are molds, while bacterial mechanisms (including spore-forming bacteria) are less typical for gingerbread and, as a rule, do not determine the shelf life under standard processing and packaging conditions. Molds are able to grow in the presence of oxygen even at relatively low values of *a_w* (down to ~ 0.62 and above), and the risk increases in packaged products due to moisture retention in the package headspace and on the product surface [6]. For gingerbread products, the presence of species adapted to reduced *a_w* values is critical. In particular, xerophilic fungi such as *Eurotium* spp. (now often considered in relation to the teleomorphic forms of *Aspergillus*) and *Wallemia sebi* have been identified in the production flow of gingerbread manufacturing; these microorganisms are associated with the spoilage of low-moisture sweet products and are able to survive technological processing conditions [5]. The resistance of xerophiles to preservatives may vary: in a model study with xerophilic isolates, propionic acid demonstrated higher effectiveness against spoilage compared with potassium sorbate, which is important when selecting fungicidal preservatives.

In fillings with higher *a_w* or acidity, osmophilic and acid-tolerant yeasts may dominate, whereas spore-forming bacteria (*Bacillus* spp.) are usually limited by the low water availability in the gingerbread matrix but may pose a risk in cases of local moisture increase or temperature control failures. Therefore, when assessing risks, the product should be considered as a multi-component system, and the parameters of individual components should be controlled separately [6, 7].

Another important aspect concerns the sources of contamination and the critical control points in the process after baking. In a study investigating the factors responsible for gingerbread spoilage, it was shown that the air of the production environment is the main source of spores, while the cooling area is characterized by

increased mycological load [7]. In samples taken from production facilities, *Aspergillus* spp. and *Penicillium* spp. predominated, whereas *Aspergillus niger* and *Penicillium chrysogenum* were isolated from finished products. Importantly, challenge tests demonstrated that *A. niger* and *P. chrysogenum* were able to grow in gingerbread, with *A. niger* showing higher enzymatic activity (amylase/protease/lipase), which may accelerate the degradation of the product structure as well as its sensory and structural quality. Thus, for gingerbread products it is not only the "presence of spores" that matters, but also which particular species contaminate the product and how capable they are of degrading its structure.

The prediction of microbiological stability is largely based on water activity and the sorption behavior of the gingerbread matrix. Studies show that the relationship between moisture content and *a_w* in gingerbread has a characteristic S-shaped form, which is typical for many products with a high sugar content. This relationship is usually described using sorption isotherms. For their mathematical representation, the GAB model (Guggenheim-Anderson-de Boer) is often applied, as it allows estimating the ability of a product to absorb or release moisture at different levels of water activity. The model provides a reliable description of moisture behavior in food systems over a wide range of storage conditions and is therefore widely used in studies dealing with product stability and shelf-life prediction [8].

Importantly, the relationship between product moisture and *a_w* is not linear. It varies depending on product temperature, storage conditions, and formulation, particularly the sugar content. This occurs because sugars are able to bind water molecules effectively, thereby reducing the fraction of water that remains available within the product structure.

Certain thresholds of environmental relative humidity may lead to a sharp increase in the equilibrium moisture content of the product, effectively marking the point at which the system shifts toward a higher risk of microbial growth. For this reason, storage parameters for gingerbread, including relative humidity and temperature, as well as the barrier properties of packaging, should be aligned with the sorption isotherm specific to the given formulation rather than transferred by analogy from other flour-based confectionery products.

In multi-component products, moisture migration is one of the main factors limiting shelf life. In filled gingerbread products, average values of moisture and water activity may conceal local conditions at the phase boundaries. Moisture migration is not always governed solely by the *a_w* gradient; differences in water-binding mechanisms and matrix composition may lead to moisture transfer patterns that deviate from simple diffusion-based predictions [9]. The direction of initial moisture transfer depends not only on the *a_w* gradient but also on the

affinity of the components for water and on the proportion of free water present. A component containing a higher fraction of free water may release moisture to another component with stronger water-binding capacity, even if their initial *a_w* values do not fully predict this transfer.

According to production studies, the average *a_w* of finished gingerbread products was approximately 0.655. However, the product is highly heterogeneous: in the gingerbread base the *a_w* can be very low (around 0.327), whereas in the fillings it may reach values close to 0.949 [7].

Packaging characteristics, particularly air and water vapor permeability, together with the relative humidity of the surrounding environment, further influence moisture exchange through the headspace inside the package. In filled gingerbread products, this means that the interface between the crumb and the filling may gradually reach higher moisture levels and *a_w* values, creating favorable conditions for the growth of microorganisms that would otherwise remain limited by the low water availability in the main gingerbread matrix.

Thus, microbiological spoilage of filled gingerbread products results from the combined influence of several factors arising from the interaction of: (i) product systems with different physicochemical properties (crumb–filling), (ii) post-baking contamination, (iii) *a_w* and sorption behavior, and (iv) internal moisture migration within the multi-component crumb–filling system.

The main factors influencing microbiological spoilage of filled gingerbread products are presented in **Fig. 12.1**.

Fig. 12.1 Key factors affecting the microbiological spoilage of filled gingerbread
Source: developed by the authors

12.3 Spoilage mechanisms and risk factors in filled gingerbread

The main form of microbiological spoilage in gingerbread is the growth of mold on the surface and in the near-surface layers. This process is often initiated by

airborne spores and by cross-contamination during post-baking handling, cooling, and packaging. In a classic study of gingerbread production, the highest concentrations of fungal spores were associated with the cooling room, conveyor areas, and the packaging line. This finding highlights the importance of the processing environment as a source of contamination, not only the raw materials [5]. A more recent survey conducted at a commercial production facility identified the cooling and packaging areas as "hot spots" of airborne microflora. Disinfection of walls and ceilings with 95% ethanol reduced fungal counts from 1.66×10^3 to 80 CFU/m^3 in the cooling room and from 1.93×10^3 to 160 CFU/m^3 in the packaging area [7].

Control of spoilage in filled gingerbread should simultaneously (i) reduce the growth potential of microflora in the filling and in the baked layer and (ii) minimize post-baking contamination and oxygen availability.

Thermal lethality is not always sufficient against xerophilic molds. For example, *Eurotium* spp. have been reported to withstand heating at 75–85°C for 60 min, whereas *Wallemia sebi* can be inactivated after 30 min at 65°C [5].

Limiting water availability after baking is therefore critical. A commonly cited practical recommendation for long-term storage at room temperature is to maintain product moisture below approximately 12–15% (on a dry matter basis), which corresponds to about 60–64% relative humidity [8]. However, as discussed above, in filled gingerbread the average moisture level may vary significantly at the crumb-filling interface, particularly when the filling contains fruit components or dairy-derived ingredients that can support the growth of yeasts and molds [7].

The thermodynamic driving force for moisture migration in multicomponent systems is the tendency of a_w to equilibrate between domains (dough/crumb-filling-surface layer). From a kinetic perspective, the rate of this process depends on the effective diffusion of water and the geometry of contact between the domains. In practice, control can therefore be achieved either by reducing the Δa_w gradient through formulation adjustments or by limiting mass transfer through structural or barrier approaches [10].

Adjustment of water activity (a_w) through formulation can be achieved in several ways.

First, this involves increasing the proportion of soluble solids such as sucrose, glucose syrup, or invert sugar. Another approach is the use of moisture-binding and moisture-retaining agents, including glycerol and sorbitol, as well as the selection of hydrocolloids capable of binding water without releasing it during storage. The addition of up to 5% glycerol derived from hydrogenated cottonseed oil has been

recommended in gingerbread formulations to improve product quality during the storage period [11].

For fillings, high-carbohydrate systems can reach inhibitory *a_w* values. Fruit-based and chocolate fillings with *a_w* values of about 0.74–0.77 and soluble solids content above approximately 65°Brix have been reported as microbiologically stable at room temperature over extended storage periods [12]. A similar heat-stable filling contained about 65% soluble solids and had a pH of 3.3–3.5, using low-methoxyl pectin to form stable gels compatible with thermal processing [13]. Such approaches simultaneously reduce *a_w*, limit bacterial growth through increased acidity, and slow moisture migration due to the presence of a structured gel network.

In products with a complex heterogeneous structure, not only the average moisture content but also the local *a_w* profiles near the crumb-filling interface are critical. Moisture redistribution in this area may create micro-zones with higher water availability. These zones are particularly susceptible to contaminant growth, especially xerotolerant molds, and are also associated with textural defects such as local softening of the crumb near the filling.

Moisture migration interacts with staling processes and related textural changes.

Recrystallization of sucrose and changes in the crystalline structure of gingerbread during storage can affect perceived hardness as well as the distribution of water within the product. The use of raffinose has been proposed as a way to reduce the intensity of sucrose crystallization and slow quality deterioration during storage [14].

In another study, replacing conventional wheat flour with waxy wheat flour improved moisture retention during storage. The experimental samples showed moisture losses of 0.08–0.18%, compared with 0.20–0.38% in the control after 25 days. The authors also suggested combining modified atmosphere packaging, freezing, and the use of antioxidants as additional approaches to extend shelf life [15].

From an engineering perspective, moisture dynamics in multicomponent products can be described using multilayer diffusion models together with the vapor permeability characteristics of the packaging material. For gingerbread products, such modelling may support the selection of appropriate barrier films and help manage humidity within the package headspace in order to prevent crumb softening or moisture accumulation that may promote mold growth.

However, in practice the most common trigger for mold development remains improper storage conditions, which create a favorable environment for fungal growth (**Fig. 12.2**).

Fig. 12.2 Surface mold growth on gingerbread (gingerbread with cherry filling – packaged in a cardboard box; third month of storage during the summer period; non-compliance with recommended temperature and humidity conditions in the retail network)
Source: author's photo

12.4 Study of microbiological spoilage of filled gingerbread

Soft gingerbread cookies filled with cherry-flavored fruit filling and coated with sugar glaze, produced by HD Bakery & Snacks ALC, Ukraine, were used in this study. These products comply with the safety requirements of the national standard of Ukraine DSTU 4187:2003 – Gingerbread Confectionery Products. The product formulation is presented in **Table 12.1**.

To investigate the possible causes of microbiological spoilage in gingerbread with cherry filling, two samples were examined: a fresh gingerbread sample (FGB) and a sample with an exceeded storage period (PGB). The latter had been stored for 120 days from the production date in the original packaging under conditions that did not meet the recommended storage regime. The recommended conditions were a temperature of (18 ± 5)°C and relative humidity not higher than 75%. During the experiment, the samples were kept under uncontrolled fluctuations of temperature (0°C ≤ T ≤ 24°C) and relative humidity (55% ≤ RH ≤ 90%). These conditions were chosen to stimulate the development of microbiological spoilage.

a_w of the gingerbread samples was measured using an AquaLab 3TE instrument. Each measurement was performed in triplicate, and the mean value was used for further analysis.

The moisture content (%) was determined by the thermogravimetric method at a drying temperature of 102 ± 2°C. The reported value represents the arithmetic

mean of two parallel measurements. The difference between parallel determinations did not exceed 0.24%.

Table 12.1 Formulation of the "Cherry Orchard" gingerbread cookies

Raw material	Quantity, kg	Formula, % (to total)
Wheat flour	475.77	43.18
Sugar	281.75	25.57
Milk powder	24.05	2.18
Sodium bicarbonate	3.07	0.28
Ammonium carbonate	2.72	0.25
Thermostable cherry filling (flavoring filler with cherry flavor)	145.76	13.23
Vanilla-cream flavoring	0.94	0.09
Glucose-fructose syrup	112.20	10.18
Improver Probake SP	0.95	0.09
Palm oil	54.50	4.95
Water	0.13	0.01
Total	**1101.84**	**100.00**

The total number of mesophilic aerobic and facultative anaerobic microorganisms was determined using non-selective nutrient medium plate count agar (PCA) according to ISO 4833. Cultivation conditions: temperature 30 ± 1°C; incubation time 72 ± 3 hours.

For the determination of coliform bacteria, the selective culture medium Violet Red Bile Lactose Agar (VRBL agar) was used according to ISO 4832.

The number of yeasts and molds was determined on Sabouraud agar with incubation at 25 ± 1°C for 5 days according to DSTU 8447:2015.

Pathogenic microorganisms, including *Salmonella* spp., were detected according to DSTU EN 12824:2004.

According to the results of our study, the water activity of both samples did not exceed the levels considered critical for the microbiological stability of food products (**Table 12.2**).

Although the data obtained in our study generally align with the trends described by other researchers, we did not find evidence of a significant difference in water activity among the different layers of the gingerbread.

However, this did not guarantee the microbiological stability of the product under improper storage conditions (**Table 12.3**).

Table 12.2 Water activity and moisture content in "Cherry Orchard" gingerbread cookies

Analyzed sample	*a_w*			Moisture, %
	Filling	Filling-crumb interface layer	Crumb	
FGB	0.728	0.721	0.718	15.09
PGB	0.669	0.637	0.625	12.68

Table 12.3 Microbiological indicators of "Cherry Orchard" gingerbread cookies

Microbiological indicator	Requirement according to DSTU 4187:2003	Time of storage, day	
		0	120
Number of mesophilic aerobic and facultative anaerobic microorganisms, CFU per 1 g of product, not more than	5.0×10^3	5.0×10	2.0×10^3
Bacteria of the coliform group	Not permitted in 0.1 g	Not detected	Not detected
Yeasts, CFU per 1 g of product, not more than	5.0×10	0	< 5
Molds, CFU per 1 g of product, not more than	5.0×10	0	0
Pathogenic microorganisms, including *Salmonella* spp., in 25 g of product	Not permitted	Not detected	Not detected

The results of the microbiological analyses confirmed that the studied product complied with the established microbiological standards throughout the entire storage period and remained safe according to the tested indicators.

No mold growth was detected during the whole storage period, indicating an appropriate sanitary condition of the production process.

However, after 120 days of storage, the presence of yeasts was detected in the product (less than 5 CFU/g, which is significantly below the permissible level of 5.0×10^1 CFU/g). These microorganisms may potentially contribute to product spoilage during further storage.

Yeast growth usually stops at *a_w* values of about 0.88–0.90. Nevertheless, osmophilic yeasts are able to grow at much lower water activity levels, down to approximately $a_w \approx 0.60–0.65$ [16].

The approximate *a_w* thresholds for the development of major groups of microorganisms can vary over a fairly wide range. Nevertheless, at $a_w \approx 0.60$, the growth of bacteria and most fungi is effectively inhibited, and only spores are able to survive. In practice, achieving such a low water activity in filled gingerbread is quite difficult. For this reason, manufacturers usually rely on a combination of technological measures to ensure product stability during storage.

12.5 Process sanitation and control of the production environment

Since contamination often occurs after baking, maintaining good hygiene in the production environment is essential. Potential sources of contamination include the air in cooling rooms, conveyors, packaging areas, and open containers used for cooling sugar solutions [5]. Practical measures to reduce this risk include separating "raw" and post-baking zones, managing airflow and filtration in cooling rooms, prioritizing dry cleaning methods (to avoid creating moist niches), minimizing the time between product exposure and packaging, and implementing validated sanitation procedures for conveyors, trays, and other contact surfaces. The effectiveness of sanitation practices should be verified through regular microbiological monitoring of air and surfaces, as illustrated by the significant reduction in airborne CFU counts after ethanol disinfection in a production facility [7].

For filled gingerbread, sanitary control should also extend to the filling preparation system, including mixing tanks, pumps, and dosing equipment. High-moisture fillings may support the survival of yeasts and molds if product residues or biofilms are present. In addition, packaging operations should be designed to limit oxygen access and the deposition of spores, for example by sealing the product promptly after cooling and selecting packaging materials with suitable barrier properties.

12.6 Chemical approaches to prevent microbial growth

Preservatives remain a common tool in products intended for extended storage, although their selection should take into account the target microflora (xerophilic or common molds), as well as the pH and a_w of the product. Potassium sorbate and sodium propionate have shown the highest antifungal activity, and their combination with chitosan was reported to reduce fungal growth by more than 80% [7].

These findings support the concept of targeted application of preservatives, for example in the filling (where a_w is higher) and/or on the product surface, where spoilage typically begins, provided that regulatory limits are respected and consumer expectations are considered.

To reduce sensory impact and improve the stability of antimicrobial agents (such as essential oils, organic acids, or enzymes), microencapsulation is increasingly considered a promising approach. Encapsulation protects active compounds during processing and allows their controlled release over time. Review studies describe

several microencapsulation strategies used in food systems, including spray drying, coacervation, lipid capsules, and polymer matrices, with the aim of protecting sensitive ingredients and controlling release kinetics [17, 18].

In filled gingerbread, encapsulated antifungal compounds may be incorporated into the filling, applied as a surface coating, or embedded into edible films, helping to maintain inhibitory concentrations during storage without causing a pronounced off-flavor or odor.

Edible coatings and biodegradable films are also considered promising solutions, as they can function both as surface barriers and as carriers of bioactive substances. Research on biodegradable films for bakery and confectionery products, using differential scanning calorimetry, has shown that edible coatings can improve product quality and enable the incorporation of bioactive compounds that would not withstand thermal processing [19].

For gingerbread products, such coatings may help reduce spore attachment, limit oxygen transfer at the surface, or deliver antifungal agents through controlled release. When combined with microencapsulation, edible films may serve as a multifunctional layer of active packaging [18, 19].

12.7 Packaging and physical treatment methods

Packaging influences product spoilage by controlling oxygen availability, moisture exchange with the surrounding environment, and the microclimate at the product surface. For low-moisture products, selecting films with low water vapor transmission rates (WVTR) helps prevent moisture uptake under conditions of high ambient humidity. At the same time, for products prone to drying and hardening, an excessively strong barrier may lead to undesirable textural effects, such as condensation, and therefore requires careful optimization. Approaches to shelf-life evaluation also show that predicted storage stability may depend significantly on the sorption isotherm model applied. For this reason, selecting appropriate packaging materials requires product-specific sorption data [20].

Non-thermal methods can be used as an additional step after baking, when the product becomes most vulnerable to secondary microbial contamination. UV-C treatment can reduce surface fungal spoilage, but its effectiveness depends on selecting an appropriate dose and ensuring adequate irradiation of the entire product surface [21]. Cold atmospheric plasma has also been reported to reduce microbial load, although overly intensive treatment may cause surface drying and lead to changes in product texture [22].

Ozonation is more commonly applied for sanitizing air and surfaces in cooling and packaging areas, where secondary contamination most often occurs. In food facilities equipped with ozone-based air treatment systems, lower bacterial and fungal counts in the air and reduced bacterial contamination on surfaces have been reported [23].

Ionizing irradiation may be useful for certain types of packaged products intended for long-term storage. However, its use is generally justified only in cases where maximum microbiological stability is prioritized over possible changes in product quality [24].

12.8 Practical strategies for improving microbiological stability of products and directions for further research

No single method can fully prevent spoilage in filled gingerbread. Effective control requires the combination of complementary "hurdles". Based on the data discussed above, a practical integrated strategy may include:

1) designing fillings with low *a_w* and/or low pH. For example, high-solids fruit gels with pH around 3.3–3.5 [12, 13];

2) the use of water-binding ingredients in the gingerbread base. For example, glycerol up to about 5%, provided technological and sensory acceptability [11], together with the selection of flour types and hydrocolloids that help reduce moisture loss and staling [15];

3) the targeted application of antifungal preservatives in critical microenvironments (filling, interface, and product surface), with the effectiveness of potassium sorbate and propionates confirmed at the production level [7];

4) minimizing post-baking contamination through zoning, sanitation, and environmental monitoring [5, 7];

5) selecting packaging materials and edible coatings based on product-specific sorption behavior and barrier requirements [8, 19, 20].

The analysis carried out indicates the need for further in-depth research on the factors determining the stability of filled gingerbread during storage. In particular, studying local profiles of *a_w* and moisture at the crumb-filling interface appears especially promising, as this zone often represents a critical area for microbial development. Measurements should therefore be performed throughout the storage period under different conditions of relative humidity and ambient temperature, which would allow a more accurate assessment of moisture redistribution within the product.

Another important direction involves mathematical modelling of moisture migration in multicomponent systems, taking into account not only the properties of the product itself but also the characteristics of the packaging. Such models should integrate formulation parameters, water activity of individual components, WVTR, and storage conditions (relative humidity and temperature). Further validation of these models with experimental data would allow better prediction of product quality changes and support the selection of appropriate packaging for specific types of gingerbread.

A promising technological approach is the use of encapsulation techniques or structured systems within the filling that can act as barriers to moisture migration. Future studies should focus on selecting suitable capsule shell compositions, evaluating their resistance to mechanical and thermal stresses during mixing, forming, and baking, and assessing permeability and structural stability of the capsules during storage of finished products.

Further research should also address the evaluation of combined hurdle strategies for ensuring microbiological stability, with particular attention to fungi of the genera Aspergillus and Penicillium, as well as xerophilic species.

In addition, the development of standardized challenge-test protocols for filled gingerbread would be useful for obtaining comparable results across different studies. Such protocols should define the selection of test microorganisms, incubation conditions, and criteria for evaluating product acceptability. The implementation of these approaches would contribute to more effective control of microbiological stability and improve the safety of filled gingerbread products.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper.

Data availability

Manuscript has no associated data.

Use of artificial intelligence statement

The authors used the AI assistant Perplexity (Grok 4.1, Perplexity AI) for translation and literature source selection. The authors bear full responsibility for the

final manuscript. Generative AI tools are not credited and are not responsible for the final results.

Authors' contributions

Sergiy Smirnov: Writing – original draft, Writing – review and editing, Investigation, Visualization, Validation.

Olesia Priss: Supervision, Conceptualization, Methodology, Writing – original draft, Investigation, Writing – review and editing, Formal analysis.

Svitlana Danylenko: Writing – original draft, Investigation, Visualization, Validation.

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CHAPTER 13

Kale as a functional vegetable. Nutritional value, bioactive compounds and the influence of processing and cultivation

Olesia Priss
Yanina Chetverikova
Viktoriia Vertegel

Abstract

Kale (*Brassica oleracea* var. *acephala*) is one of the most valuable leafy green vegetables due to its high content of important phytonutrients.

Recently, kale has attracted increasing attention from scientists as a functional product for health nutrition.

This study summarizes current knowledge about the chemical composition of kale, focusing on glucosinolates, isothiocyanates, and phenolic compounds that exert antioxidant, anti-inflammatory, and chemoprotective effects.

Particular attention is paid to the influence of biotic and controlled abiotic stresses on the accumulation of these compounds in plant tissues.

The work also examines the influence of culinary and industrial processing technologies on the preservation and transformation of biologically active substances in kale. It is summarized that heat treatment using water significantly reduces the content of glucosinolates and phenols, while steaming, short-term frying, and freezing after blanching preserve these compounds better.

Innovative non-thermal technologies, such as high hydrostatic pressure, also show potential to increase the conversion of glucosinolates to biologically active isothiocyanates.

The results highlight the importance of optimized growing and processing conditions to stabilize the nutritional and functional value of kale products. Therefore, kale can be considered a promising raw material for the development of functional health products for the prevention of diet-related chronic diseases.

Keywords

Kale, glucosinolates, isothiocyanates, phenolic compounds, cultivation, processing, nutritional quality, functional food.

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13.1 Introduction

The global trend toward a transition to healthy eating and a healthy lifestyle is gaining significant momentum. Against this background, there is a growing interest among researchers in dietary patterns as a key to combating non-communicable diseases. Expanding the diversity of plant-based products in the daily diet is an important factor in providing the human body with essential vitamins, microelements, amino acids, and other biologically active compounds that support normal physiological functions. On the other hand, the transition to diets with a predominance of plant-based components is considered an important direction in the context of sustainable food systems, as it contributes to reducing environmental pressure, rational use of resources, and improving food accessibility. At the same time, an increasing number of studies focus on expanding the range of plant crops in human diets. It is believed that out of approximately 300,000 plant species, only about 5,000 have ever been used as food, and only 150–200 have been widely used in modern diets [1]. Most of the remaining species are still underutilized and belong to the group of so-called underutilized, neglected, traditional, rare, or wild vegetables. Despite this, they have high nutritional value, are rich in biologically active compounds, and are often characterized by ecological plasticity. Therefore, according to many researchers, they should be "brought back to the plate" through promotion, domestication, and commercialization [2].

Ukraine has significant potential for underutilized, niche vegetable crops – both introduced and local – which occupy very small cultivation areas but are distinguished by their nutritional value, ornamental appeal, and market prospects. In the forest-steppe zone, atypical species have already been tested, including anguria cucumber (*Cucumis anguria*), kiwano (*Cucumis metuliferus*), okra (*Abelmoschus esculentus*), aromatic cephalophora (*Cephalophora aromatica*), lemongrass (*Cymbopogon citratus*), and chufa or tiger nut (*Cyperus esculentus*). These plants combine nutritional and spicy-aromatic value with pronounced decorative qualities, which allows them to be used both in urban landscaping and as elements of "edible landscapes" in parks and household plots [3].

Expanding the range of cultivated crops can become a key way to diversify agricultural production in times of military threats, climate change, and price fluctuations. At the same time, demand for new, niche, and leafy vegetables is growing by 30% annually [4]. This creates a reserve for increasing dietary diversity among Ukrainians, especially with regard to leafy greens, where actual consumption is 9% below the recommended dietary level [5]. Global studies and reviews show that underutilized, nutrient-rich vegetables and legumes (including traditional varieties)

help combat "hidden hunger", increase the resilience of agrosystems to drought and extreme weather, and provide small farmers with new sources of income [6, 7].

Kale (*Brassica oleracea var. acephala*) is an underutilized niche crop with exceptional nutritional and functional value, making it ideal for expanding the vegetable assortment in Ukraine. It is rich in vitamins A, C, K, minerals (calcium, iron, magnesium, potassium), dietary fiber, glucosinolates, polyphenols, carotenoids, and flavonoids, and is therefore classified among superfoods with antioxidant, anti-inflammatory, hypolipidemic, and potentially anticancer effects [8]. Studies on organically grown kale show that a 100 g serving can significantly cover the daily requirements for calcium, manganese, iron, phosphorus, and copper, and provide 5.7–8.7 g of prebiotic carbohydrates, combining mineral richness with the function of a "food for the microbiome" [9, 10]. This makes kale a promising tool for combating micronutrient deficiencies and obesity, especially in diets with low consumption of leafy vegetables.

For niche production, it is important that kale is quite adaptable to growing conditions: it can produce high biomass in open fields and greenhouses, is suitable for organic systems, vertical farming, and hydroponics; optimization of nutrition allows regulation of yield and the content of bioactive compounds. Varieties differ in morphology, productivity, and nutrient composition. European and Asian studies confirm this, providing a basis for breeding adapted forms with better keeping quality and phytonutrients. With the growing demand for organic and highly nutritious green vegetables, kale can become a competitive niche crop for small and medium-sized farms, urban and vertical farming, provided that technologies are adapted to Ukrainian conditions, local varieties are created and a consumer culture of its consumption is formed.

Many publications position kale as a superfood with antioxidant, anti-inflammatory, anticancer and antibacterial properties [10]. Kale is widely used in culinary applications. It is consumed raw in salads and "green" smoothies, added to soups, stews, stews, omelets, pan-fried dishes, used as a side dish or vegetable base, and also baked and dried in the form of "chips" as a healthy snack. In processing, kale is used for the production of frozen and canned vegetable mixes, juices and functional drinks, including fermented juices, where under the influence of lactic acid bacteria the content of polyphenols and antioxidant activity increases, while the content of antinutritional compounds decreases [11]. Leaf powder or puree is added to bread, baked goods, snacks, beverages, and soups as a functional ingredient that increases the content of dietary fiber, minerals, and bioactive compounds [12]. Kale is actively used in the development of functional products for the prevention of chronic diseases (cardiovascular, metabolic, inflammatory), in particular in experiments with fermented juice and soups intended for the elderly [13]. In addition to food applications,

kale is used to create biodegradable edible films and coatings in combination with sodium alginate, which contributes to environmentally friendly food packaging [14].

The aim of the work was to summarize current scientific data on the nutritional value and bioactive compounds of kale (*Brassica oleracea* var. *acephala*), as well as to analyze the influence of cultivation conditions, abiotic stresses and processing technologies on the accumulation and preservation of glucosinolates, isothiocyanates and phenolic compounds that determine its functional properties.

13.2 Glucosinolates and the dependence of their sweetness and content on abiotic and biotic factors

Kale is characterized by a high content of biologically active compounds – vitamins (A, C, K), minerals, polyphenols, carotenoids, chlorophylls, fiber and, above all, glucosinolates (GLS), which determine the significant antioxidant, anti-inflammatory and anticarcinogenic activity of this crop. GLS are sulfur- and nitrogen-containing thioglucosides with a single basic skeleton derived from amino acids. GLS are classified into aliphatic, indole, and aromatic groups. A wide range of total GLS content has been described for kale (2.25–93.9 μmol/g dry weight), with the ratio of indole to aliphatic compounds varying significantly depending on the variety, plant tissue, developmental stage, growing conditions and analytical method. In most edible kale varieties, sinigrin, glucoiberin and glucobrassicin dominate, often with notable contributions from progoitrin and neoglucobrassicin. Together, they can provide 70–95% of the total glucosinolates in the leaf. **Table 13.1** summarizes the approximate range of the most common individual glucosinolates in kale.

Table 13.1 Content of major glucosinolates in kale leaves

GLS class	Common GLS in kale leaves	Range, μmol/g dry wt.	Contribution to total GLS, %
Aliphatic	sinigrin	0.29–9.66	26.6
	glucoiberin	2.38–25.07	36.5
	progoitrin	0.09–2.52	3.5
	gluconapin	0.00–0.86	1.1
	glucoraphanin	0.00–1.27	1.7
Indole	glucobrassicin	0.32–17.92	24.3
	neoglucobrassicin	0.00–3.39	4.5
Aromatic	gluconasturtiin	0.00–1.38	1.8

Source: compiled by the authors based on [15–19]

In plant tissues, GLS are chemically stable. However, when cells are disrupted, the substrate is hydrolyzed by endogenous myrosinase to form isothiocyanates (ITCs), nitriles, and thiocyanates, which play a key role in plant defense against pathogens and herbivores and mediate chemopreventive effects in humans. N. Baenas reported that in kale leaves, glucoraphanin is the dominant GLS (≈ 12 mg/100 g fresh weight), and the corresponding ITC, iberin, is the main individual ITC (≈ 0.8 mg/100 g) [16]. Recent studies clearly emphasize that the preventive effects of cruciferous vegetables – anticancer, anti-inflammatory, and antioxidant – are primarily associated not with GLS themselves but with their hydrolysis products, mainly ITCs. It has been shown that ITCs, rather than GLS, are the direct chemopreventive agents that regulate tumor initiation, growth, and development in various organs [20].

Phylogenetically and morphologically distinct kale groups (German, American, Italian forms, sabellica-type variants, etc.) exhibit considerable genotypic variability in their GLS profiles: the content of individual compounds and the total GLS level in 25 cultivars vary widely, with differences between genotypes often exceeding the influence of environmental conditions [21]. Analysis of organic kale genotypes showed that even among seven samples, the content of sulfur-containing metabolites, GLS breakdown products, and associated polyphenols and carotenoids varied substantially [22]. For 30 genotypes grown in different regions, a broad range in GLS, phenolic compounds, and flavonoid content was observed; certain genotypes combined elevated GLS levels with high antioxidant activity, indicating the potential for targeted breeding for nutraceutical traits [23].

Seasonality combines changes in temperature, day length, and light intensity, so its effect on GLS in kale cannot be separated from climatic factors. In a broad set of *Brassica oleracea* forms (including kale), higher total GLS concentrations in leaves were observed in spring than in autumn; statistical analysis showed that total and indole GLS levels were explained by a combination of mean temperature, photosynthetically active radiation, and day length 2–4 weeks before harvest. Similar seasonal patterns have been observed in Chinese kale. In most cultivars, GLS content, phenolic compounds, and antioxidant activity were higher in spring and autumn than in winter, which is attributed to a more favorable combination of temperature and light and reduced stress from short days and cold [24].

Temperature acts as a moderate stressor, capable of both stimulating and suppressing GLS accumulation. In field trials, total GLS content in *Brassica oleracea* showed a negative linear but positive quadratic relationship with mean temperature two weeks before harvest. Moderate increases in temperature led to higher concentrations, whereas very low or very high temperatures caused degradation. In a "plant factory" for kale, the optimal growth temperature was 20–23°C, whereas

the maximum GLS content occurred at 14–17°C; further increases in temperature reduced their levels [25]. During cold acclimation, some cultivars (curly, lacinato) exhibited a sharp increase in aliphatic GLS (e.g., glucoraphanin > 200%), whereas in the "wild" type, cold mainly decreased glucobrassicin [26].

Light affects kale both through the daily light integral and through spectral composition and photoperiod. In a field trial with kale, cabbage, and broccoli, total leaf GLS content was positively correlated with PPF two weeks before harvest; however, at excessively high values, the curve became quadratic, indicating saturation and photostress. In artificial-light growth chambers for kale, it was shown that, under the same daily light integral, the highest GLS content occurred under a 14-hour photoperiod at moderate intensity (~ 200 µmol m^{-2} s^{-1}), whereas very long (22 h) or short (10 h) days reduced their levels [27].

Phenological stage modulates the response to seasonal and climatic factors. In the field, GLS content in kale leaves increases from the seedling stage to the onset of flowering. At the consumer stage, indole and aromatic GLS (primarily glucobrassicin) reach their maximum, whereas during the transition to the generative phase, aliphatic forms are translocated to flower buds, where, especially for sinigrin, the highest concentrations are recorded. Spring plantings reach the "consumer" stage under longer days and moderately higher temperatures, which is usually associated with higher total GLS content, whereas autumn plantings approach flowering under shorter days and lower temperatures. In sprouts, microgreens, and baby leaves under spring-summer conditions, aliphatic GLS content is generally much higher than in mature plants, but sensitivity to temperature and light at these early stages is also more pronounced.

In summary, seasonality, through the combination of temperature, day length, and light intensity, determines the "age and stage" of the plant at which the GLS profile in kale is formed. Moderately cool conditions with sufficient light and a moderate photoperiod (14–16 h) during the phase of active leaf growth favor maximal total GLS content, particularly indole GLS, whereas extreme cold or heat, excessively short or long days, and full flowering most often lead to reduced total content or a shift of the profile toward aliphatic forms.

Agronomic practices can be deliberately used as a "stress tool" to increase phenolic compounds, carotenoids, and GLS in kale, but excessive stress reduces yield and quality. Abiotic stresses (soil salinity, drought, temperature, solar radiation, phytohormones) are increasingly considered as managed technologies for bioactive compound enrichment. Controlled abiotic stresses activate signaling pathways and transcription of secondary metabolism genes, resulting in increased enzyme activity, enhanced synthesis of phenolics and GLS, and their accumulation in tissues. In kale,

such controlled stresses (particularly cold and radiation) have been shown to substantially increase the content of protective metabolites with anti-inflammatory and anticancer properties [28]. At the same time, excessive stress increases the risk of up to 50% yield loss, accumulation of antinutritional compounds (oxalates, nitrates, phytates, tannins), and deterioration of consumer quality.

The type of fertilizer and overall agronomic management shape the kale microbiota and associated risks. In South Korean farms, traditional organic fertilizers based on manure were associated with a high proportion of coliform bacteria carrying antibiotic resistance genes compared with other fertilization systems. The potential for horizontal transfer of these genes within the coliform population has been confirmed [29]. Therefore, fertilizer practices should be reconsidered as a component of food-chain biosafety.

13.3 Effect of processing technologies on glucosinolates and isothiocyanates

Thermal culinary methods differently affect GLS retention and ITCs formation. Boiling kale in water causes cell structure disruption, diffusion and leaching of GLS into the cooking water, as well as thermal degradation and rapid inactivation of myrosinase, resulting in retention of only about 20–40% of the original GLS and ITCs content in the vegetable. In contrast, steaming and stir-frying involve minimal water contact and moderate heating time, allowing at least 50% of GLS and their corresponding ITCs to be preserved in kale. Studies on the *Brassica* family consistently identify steaming as the most favorable method for preserving these compounds, whereas boiling and blanching in a large volume of water are considered the most destructive [15].

Industrial pretreatment and preservation methods also have a critical impact on GLS content in kale. Studies of fresh, blanched, boiled, frozen, canned, and dried kale leaves showed that after blanching followed by rapid freezing, total GLS content remained the highest among all storage methods even after 12 months, whereas canned samples exhibited the lowest values [30]. Frozen products from blanched material contained on average 20% more GLS than those frozen after boiling, 58% more than canned products, and nearly 50% more than dried samples. Data summarized across various vegetables indicate that the combination of "blanching-rapid freezing" is optimal for preserving both GLS and the potential amount of ITCs during subsequent consumption.

Physical processing methods, particularly high hydrostatic pressure, are considered a promising tool for managing the myrosinase–glucosinolate system without

significant deterioration of sensory qualities. For kale leaves, treatment at 600 MPa resulted in a substantial increase in myrosinase activity and the highest conversion rate of GLS to ITCs, reaching 70.4%, although the total GLS content in these samples was lower than in raw or solely thermally treated leaves [31]. Microscopic analysis revealed characteristic damage to veins, edges, and leaf surfaces, which facilitates enzyme-substrate contact and enhances ITCs formation. A review of modern non-thermal methods (high pressure, pulsed electric fields, ultraviolet irradiation) for various cruciferous vegetables generally confirms that these techniques can either increase or modify the profile of GLS and their hydrolysis products depending on processing conditions, while remaining gentler compared with conventional thermal pasteurization.

The mechanism of ITCs formation from GLS in kale is determined by a combination of factors, some of which are directly related to processing. The activity of endogenous myrosinase, which catalyzes GLS hydrolysis, is significantly reduced by intense heating, particularly during prolonged boiling or sterilization, which decreases ITCs yield in favor of nitriles and other by-products. Individual studies on various *Brassica* species have shown that changes in the pH of the reaction medium to household acidic or slightly alkaline values, as well as dilution during chopping or preparation, can sharply increase the proportion of ITCs among hydrolysis products.

A combined analysis of the data allows the formulation of a kale-processing approach aimed at maximizing GLS retention and stimulating ITCs formation. At the household level, short steaming or brief stir-frying is recommended, possibly with prior leaf chopping to activate myrosinase. In industrial settings, the most rational approach is blanching followed by rapid freezing or gentle drying, preferably via lyophilization, which ensures the highest residual GLS content after long-term storage. A promising direction is the application of high hydrostatic pressure as an alternative to thermal pasteurization for producing functional kale ingredients with increased ITCs content. Considering the pH of the medium, degree of leaf chopping, and hydrolysis conditions when making beverages, purees, and other processed kale products offers additional opportunities to deliberately increase the bioavailability of these compounds in final food systems.

13.4 Phenolic compounds

Phenolic compounds in kale form one of the key components of its antioxidant and functional potential. Approximately three dozen phenolic components have

been identified in kale, mainly flavonol glycosides of quercetin and kaempferol, as well as derivatives of hydroxycinnamic acids – p-coumaric, ferulic, sinapic, and caffeic acids.

Total flavonol content in fresh leaf tissue is about 646 mg rutin equivalents (RE)/100 g, and hydroxycinnamic acids are about 204 mg RE/100 g, totaling roughly 0.85 g phenolic compounds per 100 g fresh weight (FW). The main individual compounds are highly glycosylated acylated derivatives of kaempferol and quercetin, accounting for approximately 18–19% and 16–17% of total flavonols, respectively. After acid hydrolysis, two aglycones predominate – quercetin (~ 44 mg/100 g) and kaempferol (~ 58 mg/100 g) – indicating the dominance of their glycosides in the phenolic profile [32].

Studies of phenolic acid content in kale leaves have identified nine acids, with ferulic and caffeic acids being the main ones (totaling 4269 and 4887 ng/g FW, respectively) [33]. A significant portion of these acids occurs in bound form, contributing to cell wall protection and plant stress responses. In the red-leafed kale variety "Redbor F1", an even more diverse polyphenolic spectrum has been described –47 different glycosides of flavonols, anthocyanins, and hydroxycinnamic acids, with a total content of approximately 872 mg polyphenolic equivalents per 100 g fresh weight. Under field conditions, this hybrid exhibits about 20% higher soluble phenolics and flavonoids compared with the green cultivar Dwarf Blue Scotch, which correlates with higher antioxidant activity.

Studies of different genotypes confirm that most secondary metabolites in kale are phenolic compounds: 70–80% of identified metabolites belong to flavonoids (kaempferol and quercetin glycosides, anthocyanins), chlorogenic and other hydroxycinnamic acids, and coumarins [34]. In kale microgreens and sprouts, a wide spectrum of polyphenols is also detected, including numerous flavonoids (such as anthocyanins) and hydroxycinnamic acids, with their profile and bioavailability strongly depending on developmental stage and cultivation conditions [35].

Overall, the kale phenolic complex correlates closely with antioxidant activity, and variations by cultivar, genotype, and agronomic conditions result in a wide range of phenolic content, which is important to consider when assessing the nutritional and functional value of the raw material.

13.5 Effect of processing technologies on the phenolic compounds

Phenolic compounds in kale are highly sensitive to processing methods. Most studies show that heating in contact with water reduces total phenolic content due

to oxidation and leaching: even short boiling or blanching can substantially decrease total polyphenols in kale [36]. In kale, boiling in water caused the greatest degradation and leaching of polyphenols, whereas steaming resulted in the smallest losses, with water blanching falling in between. In another study, thermal treatments (blanching, freezing, subsequent "boil-in-bag") in green and red curly kale cultivars led to a significant reduction in total phenolics and antioxidant activity, with the red cultivar retaining phenolic compounds better [37].

At the same time, there are contrasting findings: when kale was prepared at home using short-term steaming, an increase in phenolic content (~ +86% compared with raw samples) and a simultaneous rise in antioxidant activity were observed, attributed to the softening of the cell matrix and release of bound polyphenols. For different plant parts (leaf, stem, whole plant), steaming has been shown to provide the best extraction of phenolics compared with other thermal regimes, if extraction is performed in water [38]. A comparison of steaming and sous-vide processing for the kale cultivar cv. Crispa confirmed that both methods statistically reduced total phenolic content, but losses depended on the plant part; fresh kale leaves exhibited one of the highest phenolic levels (~ 159 mg/100 g) among the organs studied [39].

A review of modern traditional and innovative cooking methods highlights that boiling and vacuum cooking in water promote phenolic losses through leaching, whereas methods with minimal water contact (steaming, microwaving, sous-vide in vacuum bags) more often preserve or even increase the extractable amount of phenolic compounds due to cell wall disruption [40].

Extrusion cooking of snacks with added fresh kale showed that high-temperature, short-time extrusion does not degrade phenolic compounds; instead, their content and antioxidant activity clearly increase with higher proportions of kale in the formulation [41]. Optimal parameters (30% fresh kale, 36% moisture) ensured maximal phenolic acid content (primarily sinapic acid) and high antioxidant activity. Similar results were obtained for corn-based snacks with 2–8% kale addition: phenolic acid content and antioxidant potential increased with the proportion of kale, and extrusion did not reduce polyphenolic activity.

In the production of frozen and canned kale, it was shown that freezing after blanching provides significantly higher residual polyphenol content after one year of storage (≈ 83–171 mg/100 g) compared with canning, where polyphenol levels were lower (≈ 91–94 mg/100 g) [42]. Reviews of kale as a functional ingredient emphasize that freezing and mild drying methods better preserve the polyphenolic profile, whereas canning with prolonged sterilization leads to substantial reductions in phenolic compound content [40].

13.6 Advantages and challenges of kale cultivation

In addition to all its health-related dietary benefits, kale is distinguished by its simple agronomic requirements. This plant tolerates adverse conditions such as high salinity, drought, and extreme temperatures allowing harvests nearly year-round in diverse climates. Kale provides multiple cuttings: older leaves can be harvested to stimulate new growth, ensuring a high total yield per area.

However, kale yield and nutritional value vary greatly depending on cultivar, cultivation system, fertilization, light, and microclimate. Conditions that maximize leaf biomass often slightly reduce the concentration of beneficial phytochemicals, and vice versa. Significant differences in yield and content of phenolics, flavonoids, anthocyanins, and carbohydrates are observed among cultivars and local populations [43].

Kale grows well on deep, moderately acidic to neutral soils (pH ~ 6–7.5) with high organic matter and uniform moisture. Proper selection of sowing dates allows maximization of yield and economic return without significant quality losses [44]. Fertilization systems and biostimulatory stresses (salinity, temperature) can enhance phenolic, carotenoid, and GLS content, thereby strengthening the health-promoting properties of the produce.

Kale responds well to organic fertilizers (compost, black soldier fly larvae frass), which increase yield and dry matter content while simultaneously reducing pest populations compared with mineral fertilizers [45]. High yields and enhanced vitamin C and other bioactive compound content can be achieved in both soil-based and soilless systems (hydroponics, aquaponics, vertical "multi-layer" beds) compared with conventional soil cultivation. In temperate climates, protective structures (high and low tunnels with mulching) allow successful winter cultivation of kale [46].

In open fields and greenhouses, kale produces the greatest biomass due to high natural light and lower planting density; in growth chambers, biomass is lower, though leaves are thinner (high leaf area per gram) [47]. Organomineral fertilizers provide the highest productivity for curly kale compared with purely organic or unfertilized systems.

Despite its hardiness, kale is highly susceptible to diseases and pests, particularly in intensive and protected systems. In soilless greenhouses, significant losses (> 70%) have been reported due to stem rots caused by the fungus Agroathelia delphinii; the risk is increased by non-sterilized organic substrates (rice straw, coconut husk, etc.). Organic production makes it more difficult to control diseases and extend postharvest life; organic kale generally has a shorter shelf life, increasing food losses.

In open fields, clubroot (*Plasmodiophora brassicae*) is a major concern: on acidic, waterlogged soils with high Al^{3+} content and with prolonged monoculture, the

disease spreads extensively, reducing yield and impairing plant nutrition. In urban cultivation, powdery mildew and black rot often dominate; warm and humid conditions, high planting density, and intensive cultivation exacerbate their impact, while straw mulching has variable effects on these diseases [48].

Among pests, aphids, cabbage whitefly, and other insects reduce yield, quality, and glucosinolate content in leaves. Minimizing losses requires integrated protection systems, cultural practices (crop rotation, timing, soil-cover materials), biocontrol, and rigorous sanitation of equipment and planting material.

13.7 Conclusion

Kale is considered a truly promising leafy green vegetable crop with high nutritional and functional value. It is distinguished by a rich complex of biologically active compounds, particularly GLS, ITCs, and phenolic substances. These components determine the pronounced antioxidant, anti-inflammatory, antibacterial, and potential anticancer properties of this crop, which underlines its important role in the formation of a healthy diet.

It has been established that the accumulation of bioactive compounds in kale tissues largely depends on the genotype, environmental growing conditions, and the influence of abiotic factors. The application of controlled stress factors and the improvement of agronomic practices make it possible to regulate the level of secondary metabolites, thereby creating opportunities to enhance the biological value of the final products.

An important factor in preserving the functional properties of kale is the selection of appropriate culinary and technological processing methods. It has been demonstrated that intensive thermal treatment in large volumes of water leads to degradation of 60–80% of glucosinolates and phenolic compounds.

At the same time, steaming, short-term stir-frying, and the use of modern processing technologies contribute to the maximum retention of nutrients. Therefore, the integrated optimization of cultivation conditions and processing regimes can become a strategic approach to stabilizing bioactive compounds in kale as a raw material for the production of functional foods.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper.

Data availability

Manuscript has no associated data.

Use of artificial intelligence statement

The authors used the AI assistant Perplexity (Grok 4.1, Perplexity AI) for translation and literature source selection. The authors bear full responsibility for the final manuscript. Generative AI tools are not credited and are not responsible for the final results.

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Authors' contributions

Olesia Priss: Supervision, Conceptualization, Methodology, Writing – original draft, Project administration.

Yanina Chetverikova: Writing – original draft, Writing – review and editing, Investigation.

Viktoriia Vertegel: Writing – original draft, Investigation, Formal analysis, Validation.

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CHAPTER 14

Frozen desserts formulated with plant-based milk: a comprehensive quality analysis

Igor Dudarev
Tamara Sydoruk
Mykola Andrushchenko

Abstract

The consumption of dairy alternative products is increasing worldwide, particularly in the frozen dessert sector, where dairy ingredients are partially or completely replaced with plant-based alternatives. Plant-based milks, including soy, rice, coconut, cashew, hazelnut, peanut, hemp, and lupine milk, are increasingly incorporated into formulations. This substitution not only replaces animal proteins and fats with plant-based ones but also enriches products with bioactive compounds from plant raw materials or their processing by-products. Analysis of the nutritional value and key physicochemical properties of these desserts shows considerable variability depending on ingredient composition and production technology, including the methods used to process raw materials. Nonetheless, it is important that such frozen desserts maintain properties comparable to conventional dairy ice cream.

The key indicators characterizing the properties of frozen desserts were grouped into the following categories: nutritional, chemical, physical, sensory, functional, and microbiological. These indicators reflect the overall quality of frozen desserts. The grouping of indicators enabled the development of a property tree for frozen desserts. To assess their quality, a quality index was proposed, which comprehensively accounts for both the weighting of the indicator groups and the individual weight of each indicator within a group. Calculation of the quality index using the developed mathematical model facilitates the objective comparison of the quality of different products, taking into account the recommended (optimal) values of the indicators. The model can also be expanded to include additional indicators that characterize product quality.

Based on the SWOT analysis of frozen desserts containing plant-based milk, strategies for product improvement were proposed, considering their strengths and

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weaknesses, as well as the opportunities and threats. The analysis was multi-criteria, covering economic, marketing, social, technological, and quality-related characteristics of the frozen desserts. The proposed framework enables a comprehensive assessment of the quality of frozen desserts formulated with plant-based milk and supports the identification of directions for their improvement and market positioning.

Keywords

Frozen dessert, plant-based milk, ice cream, dairy alternative, food quality index, SWOT analysis, property tree, bioactive compounds, mathematical model of product quality.

14.1 Introduction

Traditional ice cream is a popular frozen dessert made from dairy ingredients such as milk and cream combined with natural or artificial sweeteners [1]. A typical ice cream formulation contains approximately 10% fat, 9–12% milk solids-non-fat, 14–17% sweeteners, 0.1–0.3% stabilizers, 0.1–0.2% emulsifiers, and 60–65% water [2]. Fat contributes to a creamy texture, foam stability, flavor enhancement, structural stability, and resistance to melting. Milk solids-non-fat provide proteins, lactose, and minerals that support emulsification, air stabilization, and water-binding capacity. Sweeteners impart sweetness, while stabilizers and emulsifiers enhance structure stability and improve air incorporation. Water serves as the continuous phase in which the ingredients are dissolved and dispersed [2]. Due to its compositional characteristics, ice cream is also considered a relatively high-energy food product [3]. However, commercial ice cream generally contains low levels of natural antioxidants, including vitamin C, pigments, and polyphenolic compounds. Consequently, improving its nutritional value through the incorporation of ingredients such as fruits, vegetables, nuts, pulses, and dietary fibers – rich sources of natural antioxidants, vitamins, and colorants – has attracted increasing attention in response to growing consumer demand for healthier food products [4].

Ice cream production involves several main stages: blending or mixing, pasteurization, cooling, homogenization, aging, aeration with freezing, and hardening [5]. The processes and equipment used in ice cream manufacturing depend on the scale of production, whether industrial (large-scale plants) or artisanal (small-scale or homemade production) [6]. Dairy ingredients, sweeteners, stabilizers, emulsifiers, and flavorings are carefully measured and mixed to obtain a uniform mixture. The ice cream mix is then pasteurized at a specific temperature for a defined period, followed by rapid cooling. During pasteurization, harmful pathogenic and spoilage

microorganisms are inactivated, and hydrolytic enzymes are neutralized. The next stage is homogenization, during which a stable emulsion is formed, resulting in improved texture, consistency, and mouthfeel of the final product. Subsequently, the mixture undergoes an aging process at 4°C for 4–24 h, which prepares the mix for freezing and facilitates the partial crystallization of milk fat. During aeration with freezing, the mixture is whipped, air is incorporated, and ice crystals are formed. Finally, the ice cream is hardened by blast freezing [7].

However, the consumption of dairy-based ice cream may pose health concerns, including lactose intolerance and hypercholesterolemia [8]. Increasing concerns about sustainability and health have led more consumers to choose plant-based products, including frozen desserts made from grains, legumes, nuts, seeds, and fruits [9]. Non-dairy frozen desserts available on the market include water ices, plant-protein-based products (such as those derived from soybeans, coconuts, rice, and almonds), and fruit-based products such as sorbets, ice lollies, and smoothies [10]. Non-dairy frozen desserts are typically consumed occasionally rather than on a daily basis, with intake generally increasing during warm or hot seasons in many countries [10]. For such products, in which dairy ingredients are fully or partially replaced by plant-based raw materials, various terms are used, including plant-based ice cream (e.g., peanut ice cream, mung bean ice cream) [11], frozen desserts (e.g., frozen blueberry-soy dessert) [12], and ice cream with a combined composition. The specific name often depends on national legislation and local traditions regarding product classification and labeling.

Frozen dessert formulations incorporate plant-based milk. Plant-based milks are rich in a variety of bioactive compounds that contribute to the development of functional products. For instance, soy milk provides isoflavones and phytosterols, almond milk is a source of tocopherol and arabinose, oat milk contains β-glucans, and hemp seed milk is characterized by a high content of polyunsaturated fatty acids and essential fatty acids [13]. Bioactive enrichment of frozen desserts can be achieved through the incorporation of ingredients such as propolis [14] as well as psyllium and pectin fibers [1].

The incorporation of plant-based milk into frozen dessert formulations influences their nutritional, physicochemical, and sensory properties. Differences in the protein and fat content of plant-based milk significantly influence the texture, ice crystal formation, and overall consistency of frozen desserts [1].

The high dietary fiber content of hazelnuts can enhance the textural properties of frozen desserts due to their water-holding, gel-forming, thickening, and texturizing capacities [15]. Frozen desserts formulated with hemp milk have been reported to exhibit higher fat content, resulting in increased hardness [1]. However, these

desserts may also present a slightly undesirable aroma characteristic of hemp seed milk [1]. An increase in soy milk content has been associated with reduced sensory acceptability due to the presence of a characteristic beany flavor [16]. Despite the lower total solids content in non-dairy formulations based on coconut milk, these frozen desserts exhibit a finer texture [17]. Additionally, frozen desserts containing hazelnut milk demonstrate a smoother structure and improved melt-in-the-mouth properties, which can be attributed to reduced ice crystal formation [15].

Reducing the consumption of animal-based products is considered an effective strategy for addressing environmental concerns. Growing awareness of animal welfare, environmental pollution, milk protein allergies, and lactose intolerance, along with the increasing adoption of vegetarian and vegan diets, has led to rising interest in dairy alternatives and has strengthened their perception as sustainable food options [18]. Given the rapid development of the market for such products and the expansion of their range, it is important to analyze these products in terms of their nutritional value as well as their physicochemical and sensory properties. Such analysis is necessary to identify their main advantages and disadvantages, as well as opportunities for further development in this field.

14.2 Properties of frozen desserts containing plant-based milk

14.2.1 Nutritional value

Table 14.1 presents the nutrient composition of frozen desserts formulated with plant-based milk, including hemp, soy, rice, coconut, cashew, hazelnut, peanut, and lupine. In the analyzed formulations, dairy ingredients were either completely replaced with plant-based milk or partially substituted.

In frozen desserts formulated with plant-based milk, protein content varies widely, ranging from 1.09 to 12.12% (**Table 14.1**). In most samples, protein levels do not exceed 5.0%, whereas only certain desserts made with lupine, coconut, and hazelnut milk exhibit higher protein contents of 8.10, 10.70, and 12.12%, respectively. In contrast, some frozen desserts prepared with almond, coconut, and rice milk show very low protein levels of 1.09, 1.30, and 1.38%, respectively. Protein content primarily depends on the composition of the desserts, particularly the proportion of any dairy ingredients and the protein content of the plant-based milk used. Analysis indicates that even when plant-based milks derived from high-protein sources – such as soybeans, peanuts, almonds, lupine, hemp, and cashews – are utilized, the protein content in the final frozen desserts can remain low. This is largely due to the

processing methods of the plant-based milk and its proportion in the dessert formulation. For comparison, conventional dairy ice cream contains an average of 4.1% protein [28]. Therefore, certain frozen desserts formulated with plant-based milk can serve as a dietary source of plant protein. Although protein plays an important role in the development of ice cream structure, levels above 4% may negatively affect product quality, particularly by worsening sensory properties, increasing hardness, and reducing overrun [29].

Table 14.1 Nutritional profile of frozen desserts containing plan-based milk

Frozen dessert	Protein, %	Fat, %	Carbohydrates, %	Dietary fiber, %
AlFD	1.09–4.93	1.74–2.67	n.d.	n.d.
HeFD	2.04–3.94	3.20–9.80	16.00	0.20
SoFD	2.80–5.26	2.45–5.80	9.52–24.65	1.15–1.19
RiFD	1.38–4.20	3.32–3.98	26.61–27.4	0.14–0.30
CoFD	1.30–10.70	5.00–11.60	10.78–11.34	n.d.
CaFD	2.18–4.60	2.07–10.46	10.31–31.08	0.08–2.54
HaFD	4.38–12.12	2.73–8.02	n.d.	n.d.
PeFD	2.32–2.80	10.30–10.50	n.d.	n.d.
LuFD	2.13–8.10	1.22–10.30	20.28–21.38	0.13–0.30

Source: [1, 4, 8, 13, 15–27]
Note: AlFD – frozen dessert with almond milk; HeFD: frozen dessert with hemp milk; SoFD: frozen dessert with soy milk; RiFD: frozen dessert with rice milk; CoFD: frozen dessert with coconut milk; CaFD: frozen dessert with cashew milk; HaFD: frozen dessert with hazelnut milk; PeFD: frozen dessert with peanut milk; LuFD: frozen dessert with lupine milk; n.d.: no data

Fat is an important structural component of ice cream, as it contributes to the stability of the air phase and provides sensory attributes of the product [30]. In traditional dairy ice cream, the average fat content is approximately 16.0% [28], while in some countries a minimum milk fat content of 10% is required for products labeled as ice cream [30]. In the analyzed samples of frozen desserts formulated with different plant-based milks, the fat content ranges from 1.22 to 11.60% (**Table 14.1**). Reducing the fat content of a product is consistent with modern trends in healthy eating and contributes to a lower caloric value, since fat provides more energy per gram than other macronutrients. In desserts formulated with almond milk, the fat content is among the lowest, ranging from 1.74 to 2.67% [1, 18], although almonds themselves are a raw material with a high fat content. In contrast, certain frozen desserts formulated with coconut, peanut, and cashew milk contain relatively high fat levels

of 11.60, 10.50, and 10.46%, respectively. This fat content is mainly determined by the product formulation and the method used to produce plant-based milk from raw materials containing a significant proportion of fat.

Carbohydrates represent the most abundant macronutrient in frozen desserts, with contents ranging from 9.52 to 31.08% (**Table 14.1**). For comparison, the average carbohydrate content in plain dairy ice cream is 20.7% [28]. The highest carbohydrate levels were observed in frozen desserts formulated with rice milk, ranging from 26.61 to 27.45% [22], which is associated with the naturally high carbohydrate content of rice and, consequently, rice milk. To a large extent, the carbohydrate content is determined by the amount of added sugar in the formulation, which is used to sweeten the product and impart flavor. In addition to sucrose, other sweeteners commonly used in frozen dessert formulations include dextrose, corn syrup, invert sugar, lactose, fructose, and malt syrup. Sucrose is the most widely used sweetener; however, recent studies have focused on developing ice cream with a low glycemic index while maintaining physicochemical properties and sensory quality similar to those of sucrose-sweetened ice cream [31]. The recommended sugar content in ice cream is 14–16% [32].

Dietary fiber is widely used as a fat replacer in ice cream formulations. The incorporation of dietary fiber significantly increases the viscosity of the ice cream mix and contributes to lower melting temperatures. Ice cream produced with inulin has been reported to be comparable to full-fat products in terms of quality. Ice cream containing oat fiber exhibits increased hardness, higher freezing point temperatures, and reduced overrun compared with products without fiber incorporation [33]. The addition of plant-based milk to frozen dessert formulations also contributes to an increase in dietary fiber content. In the analyzed frozen desserts, the fiber content ranges from 0.14 to 2.54% (**Table 14.1**), with the highest level observed in one of the samples formulated with cashew milk.

14.2.2 Physicochemical properties

Table 14.2 presents the physicochemical properties of frozen desserts containing plant-based milk, including total solids, ash content, pH, specific gravity, and acidity.

The total solids content significantly influences the sensory properties of frozen desserts, particularly texture, as well as hardness, melting rate, and heat shock resistance. The total solids content of the product depends on the composition of its ingredients, especially the plant-based milk used. In addition, sweeteners (e.g., sucrose), mineral fortifiers, stabilizers, and emulsifiers also contribute to the total

solids content of ice cream. In the analyzed frozen desserts, the total solids content varies widely, ranging from 17.9 to 40.8% (**Table 14.2**), depending on the product formulation. The lowest total solids content (17.9%) is observed in a sample formulated with cashew milk, whereas samples formulated with peanut milk exhibit the highest values, ranging from 39.9 to 40.8% [25]. In plain dairy ice cream, the total solids content typically ranges from 33.9 to 44.1% [34]. Therefore, plain ice cream generally contains higher levels of total solids than products formulated with plant-based milk.

Table 14.2 Physicochemical properties of frozen desserts containing plan-based milk

Frozen dessert	Total solids, %	Ash content, %	pH	Specific gravity, g/cm^3	Acidity, %
AlFD	30.6	1.27	6.10–7.42	1.09	n.d.
HeFD	29.9–35.5	0.50–0.70	3.80–6.78	n.d.	n.d.
SoFD	24.6–34.2	0.58–1.77	6.59–7.50	0.60–0.63	0.15–0.94
RiFD	32.9–35.2	0.31–0.40	6.74–7.31	n.d.	n.d.
CoFD	22.0–35.4	0.46–0.59	5.78–6.59	0.98–1.06	0.26
CaFD	17.9–37.3	0.62–1.85	5.20–7.00	1.01–1.09	0.27–0.80
HaFD	31.4–36.8	1.15–1.30	6.19–6.61	n.d.	n.d.
PeFD	39.9–40.8	n.d.	6.71–6.74	n.d.	0.11–0.12
LuFD	33.5–39.0	0.77–1.18	6.38–7.15	n.d.	0.70–0.72

Source: [1, 4, 13, 15–26, 36–38]
Note: n.d. – no data

In frozen desserts formulated with plant-based milk, the ash content ranges from 0.31 to 1.85% (**Table 14.2**). The lowest ash content (0.31%) is observed in a sample of frozen dessert formulated with rice milk, whereas a sample containing cashew milk exhibits the highest ash content (1.85%). Ash content depends on the mineral composition of the ingredients used in the formulation. The inclusion of plant-based milk contributes to an increase in the ash content of the final product. For comparison, the ash content in low-fat ice cream is approximately 1.5% [35].

Flavored ice cream typically has a pH ranging from 3.49 to 7.32 [34], whereas frozen desserts formulated with plant-based milk exhibit a pH range of 3.80 to 7.50 (**Table 14.2**). The lowest pH value (3.80) is observed in a sample of frozen dessert formulated with hemp milk, while the highest value (7.50) is recorded for a sample containing soy milk. The acidity of frozen desserts is influenced by the ratio of dairy to plant-based ingredients, particularly in products with a combined formulation. It also depends on the composition of the plant-based

milk, as well as the presence and proportion of the fruit and berry components in the formulation.

The specific gravity of frozen desserts depends on their composition, particularly the content of total solids and incorporated air. In the analyzed samples, the specific gravity ranges from 0.60 to 1.09 g/cm^3 (**Table 14.2**). The lowest specific gravity (0.60–0.63 g/cm^3) is observed in frozen desserts formulated with soy milk. In contrast, samples formulated with almond and cashew milk exhibit higher specific gravity values, ranging from 1.01 to 1.09 g/cm^3. Therefore, the specific gravity of some frozen dessert samples exceeds that of low-fat ice cream (1.068 g/cm^3) [35].

Acidity of frozen desserts containing plant-based milk ranges from 0.11 to 0.94% (**Table 14.2**). Desserts containing peanut milk exhibit the lowest acidity (0.11–0.12%) [25]. In contrast, some samples formulated with soy, cashew, and lupine milk show higher acidity values of 0.94, 0.80, and 0.72%, respectively. Acidity significantly affects the sensory properties of frozen desserts. Optimal acidity enhances the intensity of taste and aroma, whereas both lower and higher values may lead to a deterioration in the overall sensory quality of the product.

14.3 A mathematical approach to comparative quality evaluation of frozen desserts

The properties that characterize the quality of frozen desserts formulated with plant-based milk can be classified into the following groups: nutritional (protein, fat, carbohydrates), chemical (total solids, ash, pH, acidity), physical (specific gravity, viscosity, overrun, hardness, melting rate), sensory (appearance, taste, aroma, consistence, color), functional (vitamins, minerals, dietary fiber, flavonoids, pectins, carotenoids, omega-3 and omega-6, amino acids), and microbiological (mesophilic aerobic and facultative anaerobic microorganisms, etc.). Each group is defined by a specific set of indicators, as presented in **Fig. 14.1**. This representation of product properties is referred a property tree.

As a result of evaluating the significance of these indicators by seven experts using the methodology described in [39], weighting coefficients were determined for both the groups and the individual indicators. The weighting coefficients for the groups are as follows: nutritional indicators – $m_1 = 0.211$; chemical indicators – $m_2 = 0.061$; physical indicators – $m_3 = 0.157$; sensory indicators – $m_4 = 0.252$; functional indicators – $m_5 = 0.122$; microbiological indicators – $m_6 = 0.197$. Thus, according to expert assessment, sensory indicators represent the most significant group in evaluating the quality of frozen desserts, whereas chemical indicators are the least significant.

The weighting coefficients of the indicators within each group are presented in **Table 14.3**. Among the nutritional indicators, protein content has the highest importance (m_{11} = 0.452), while among the chemical indicators, total solids content is the most significant (m_{21} = 0.400). Overrun is the most important physical indicator of frozen desserts (m_{33} = 0.324). Among the sensory indicators, taste has the highest weighting coefficient (m_{42} = 0.305), whereas color has the lowest (m_{45} = 0.086). Within the group of functional indicators, vitamin content (m_{51} = 0.218) and mineral content (m_{52} = 0.155) are the most significant.

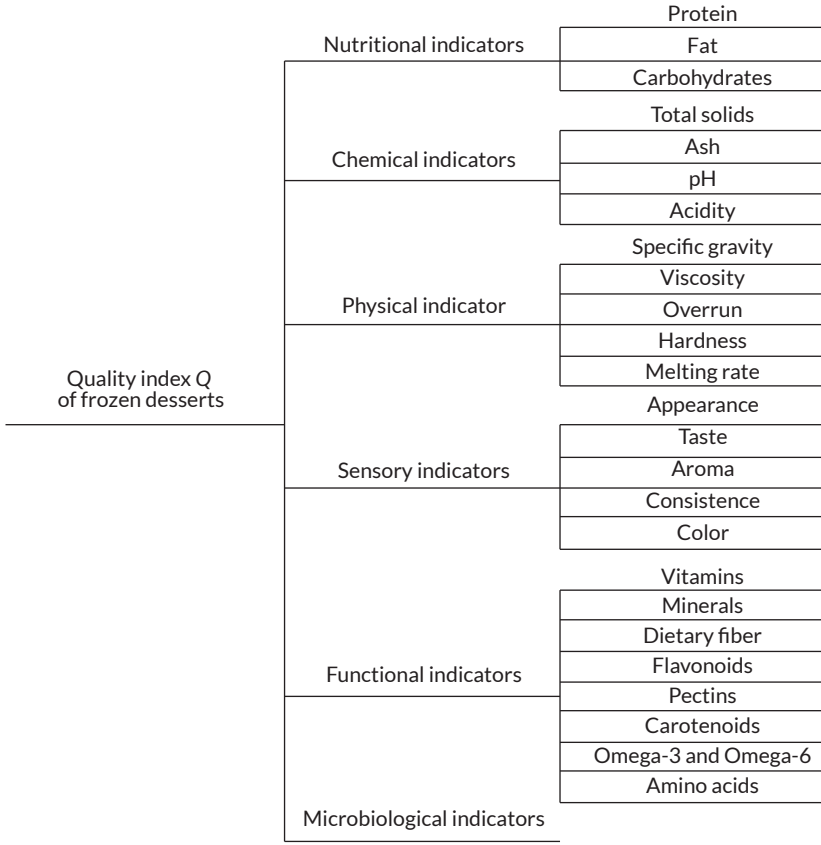

Fig. 14.1 Groups of specific indicators characterizing the quality of frozen desserts (property tree)

Table 14.3 Weighting coefficients of quality indicators for frozen desserts formulated with plant-based milk

Quality indicators	Weighting coefficients	Quality indicators	Weighting coefficients
Nutritional indicators		**Physical indicator**	
Protein	$m_{11} = 0.452$	Specific gravity	$m_{31} = 0.114$
Fat	$m_{12} = 0.262$	Viscosity	$m_{32} = 0.152$
Carbohydrates	$m_{13} = 0.286$	Overrun	$m_{33} = 0.324$
Chemical indicators		Hardness	$m_{34} = 0.181$
Total solids	$m_{21} = 0.400$	Melting rate	$m_{35} = 0.229$
Ash	$m_{22} = 0.186$	**Functional indicators**	
pH	$m_{23} = 0.257$	Vitamins	$m_{51} = 0.218$
Acidity	$m_{24} = 0.157$	Minerals	$m_{52} = 0.155$
Sensory indicators		Dietary fiber	$m_{53} = 0.119$
Appearance	$m_{41} = 0.209$	Flavonoids	$m_{54} = 0.087$
Taste	$m_{42} = 0.305$	Pectins	$m_{55} = 0.091$
Aroma	$m_{43} = 0.171$	Carotenoids	$m_{56} = 0.060$
Consistence	$m_{44} = 0.229$	Omega-3 and Omega-6	$m_{57} = 0.131$
Color	$m_{45} = 0.086$	Amino acids	$m_{58} = 0.139$

The quality index of frozen desserts formulated with plant-based milk is calculated using the following equation

$$Q = m_1 \sum_{i=1}^{3} m_{1i} q_{1i} + m_2 \sum_{j=1}^{4} m_{2i} q_{2i} + m_3 \sum_{k=1}^{5} m_{3k} q_{3k} + m_4 \sum_{l=1}^{5} m_{4l} q_{4l} + m_5 \sum_{t=1}^{8} m_{5t} q_{5t} + m_6 q_6,$$

where Q – the quality index of frozen desserts; m_1, m_2, m_3, m_4, m_5, m_6 – the weighting coefficients of the groups of indicators; m_{1i}, m_{2j}, m_{3k}, m_{4l}, m_{5t} – the weighting coefficients of individual indicators within each groups (**Table 14.3**); q_{1i}, q_{2j}, q_{3k}, q_{4l}, q_{5t}, q_6 – the relative quality indicators of the product.

Relative quality indicators of the product are calculated using the equations:

$$q^{*} = \frac{P}{P_{rec}} \text{ or } q^{**} = \frac{P_{rec}}{P},$$

where P – the measured value of a frozen dessert indicator (e.g., vitamin content, protein content, total solids content, taste score, overrun, specific gravity, etc.); P_{rec} – the recommended (optimal) value of the frozen dessert indicator.

The relative quality indicator q^* is calculated when an increase in the value of the indicator P has a positive effect on product quality. For example, if the taste of the product is rated 4 points on a 5-point scale, the corresponding relative indicator for taste is $q^* = q_{42} = 4/5 = 0.8$. The relative quality indicator q^{**} is calculated when an increase in the value of the indicator P has a negative effect on product quality. For instance, if the number of mesophilic aerobic and facultative anaerobic microorganisms in 1 g of the product is $0.8 \cdot 10^5$ CFU, while the permissible limit is $1 \cdot 10^5$ CFU, then $q^{**} = q_6 = 1 \cdot 10^5 / (0.8 \cdot 10^5) = 1.25$.

The quality index Q is useful for comparing different products, providing an objective and comprehensive numerical assessment. A product with the higher Q value is considered to have superior quality.

14.4 SWOT analysis of frozen desserts containing plant-based milk

A multi-criteria SWOT analysis was conducted to evaluate frozen desserts formulated with plant-based milk [40]. Specifically, the economic, marketing, social, technological and quality-related strengths and weaknesses of these products, as well as the associated opportunities and threats, were assessed.

14.4.1 Strengths of frozen desserts containing plant-based milk

The strengths of frozen desserts formulated with plant-based milk can be categorized as follows:

S1. Economic-related strengths: the possibility of utilizing low-cost local raw materials; affordable product pricing when using locally sourced ingredients; the ability to use different types of plant-based raw materials throughout the year, taking into account their seasonal availability; the potential use of by-products (e.g., okara) generated during plant-based milk production; and potential for cost optimization through simplified storage and longer shelf life of certain plant-based ingredients (e.g., plant-based milk powders).

S2. Marketing-related strengths: alignment with healthy eating trends; the possibility of positioning the product as vegan; the potential for marketing it as a low-calorie product free from animal-derived ingredients; the expansion of the frozen dessert assortment; and opportunities for clean-label positioning.

S3. Social-related strengths: support for healthy eating concepts; suitability for individuals with milk protein allergies and lactose intolerance; promotion of

plant-based products; and increasing consumer interest in environmentally friendly lifestyles (e.g., sustainable consumption patterns, reduced environmental footprint).

S4. Technological-related strengths: processing methods that do not significantly differ from plain ice cream production; the possibility of using various types of plant-based milk in the formulations; the use of powdered plant-based milk; the potential to combine different types of plant-based milk or both plant- and animal-based ingredients; flexibility in formulation design to tailor texture, flavor, and nutritional profile; and the potential to develop products containing functional ingredients (e.g., probiotics, dietary fiber, bioactive compounds).

S5. Quality-related strengths: a wide range of flavors; a texture comparable to that of plain dairy ice cream; the presence of dietary fiber; lower fat content; reduced caloric value; the potential for enrichment with bioactive compounds derived from plant-based ingredients; in most cases, the absence of lactose; and, in some instances, the products with specific functional or health-promoting properties.

14.4.2 Weaknesses of frozen desserts containing plant-based milk

The weaknesses of frozen desserts formulated with plant-based milk can be grouped into the following categories:

W1. Economic-related weaknesses: the need to store plant-based raw materials due to seasonal production or processing cycles; some types of plant-based milk (e.g., almond, peanut, and cashew milk) are more expensive than cow's milk; potential additional costs for imported raw materials (e.g., coconuts, almonds, peanuts); and instability in the supply of imported ingredients.

W2. Marketing-related weaknesses: consumer preference for dairy products; the requirement for extensive advertising campaigns to communicate the benefits of plant-based frozen desserts; and challenges in building consumer trust in new products.

W3. Social-related weaknesses: prejudiced attitude towards plant-based dairy analogues; and conservatism among certain consumer segments.

W4. Technological-related weaknesses: in some cases, the need to use stabilizers and emulsifiers to achieve the desired texture; the need to incorporate flavors to mask undesirable aromas and tastes from certain raw materials (e.g., hemp seeds, legumes); and the need to add colorants to achieve an appealing appearance.

W5. Quality-related weaknesses: the potential presence of allergens (e.g., soybeans, nuts); undesirable beany or atypical flavors; in some cases, a texture inferior to dairy ice cream; and unconventional coloration depending on the plant-based ingredients used.

14.4.3 Opportunities for frozen desserts containing plant-based milk

The opportunities for frozen desserts formulated with plant-based milk can be organized into the following categories:

O1. Economic-related opportunities: wide availability of plant-based raw materials; potential for expanding the market for products derived from plant-based ingredients; opportunities to export products to countries with higher demand for frozen plant-based desserts; and increasing consumer demand for functional and health-promoting products.

O2. Marketing-related opportunities: the potential to develop new brands of healthy desserts; growing popularity of vegan products; expansion of the functional dessert ranges through diverse combinations of raw materials; and promotion of products via eco-markets, social media platforms, and targeted marketing campaigns.

O3. Social-related opportunities: a growing population with lactose intolerance; increasing consumer interest in ecological and ethical food consumption; the spread of vegetarian diets; and enhanced consumer awareness of sustainable lifestyles.

O4. Technological-related opportunities: development of plant-based milk production methods that minimize nutrient losses; potential use of plant-based substitutes for animal fats, particularly carbohydrate-based alternatives; innovation in stabilizers and texturizers to improve product quality; application of advanced homogenization techniques to enhance texture and mouthfeel.

O5. Quality-related opportunities: the use of organic plant raw materials; development of novel flavor combinations; creation of functional frozen desserts enriched with vitamins, minerals, and bioactive additives; and the potential for clean-label products.

14.4.4 Threats for frozen desserts containing plant-based milk

The threats for frozen desserts formulated with plant-based milk can be organized into the following categories:

T1. Economic-related threats: fluctuations in the prices of plant-based raw materials due to crop failures and increasing costs of energy and fertilizers; economic instability that reduces consumer purchasing power; high competition in the ice cream and frozen dessert market; and dependence on imported raw materials in some regions.

T2. Marketing-related threats: instability of food trends; the possibility of product imitation by competing manufacturers; and market saturation with similar plant-based products.

T3. Social-related threats: possible negative perception of non-dairy alternatives to traditional products; and aging populations in many countries, which tend to be more conservative in their product choices.

T4. Technological-related threats: technological challenges associated with the use of novel raw materials.

T5. Quality-related threats: possible inconsistency in product properties compared to traditional dairy products.

14.4.5 SWOT-based strategic development for frozen dessert formulated with plant-based milk

SO Strategy: use healthy eating trends, lactose-free properties, and formulation flexibility (S2, S4, S5) to meet the growing demand for functional and plant-based products (O1, O2, O5). This strategy involves developing functional, clean-label frozen non-dairy desserts enriched with bioactive compounds, vitamins, and dietary fiber.

SO Strategy: use the availability of diverse and cost-effective local plant-based raw materials and by-products (S1, S4) to broaden the assortment of non-dairy frozen desserts (O1, O2). This strategy involves expanding the product range through the development of new flavor combinations based on local raw materials, thereby enhancing market competitiveness and supporting local agricultural systems.

WO Strategy: use advances in processing technology and ingredient innovation (O4, O5) to address shortcomings related to undesirable flavors and non-traditional textures (W4, W5). This strategy involves applying novel stabilizers, enhanced homogenization techniques, and optimized plant-based milk production methods to improve product quality and consumer acceptance.

ST Strategy: use strengths such as vegan positioning, environmental benefits, and functional properties (S2, S3, S5) to mitigate threats related to market competition and product imitation (T1, T2, T3). This strategy focuses on developing a strong brand identity based on sustainability, health benefits, and ethical consumption, thereby ensuring a lasting competitive advantage.

14.5 Conclusions

Given the increasing prevalence of diets among consumers that involve the partial or complete replacement of animal-based foods with plant-based

alternatives, as well as the growing market demand for functional products, the development of novel products that align with these trends is highly relevant. Among such products are frozen desserts formulated with plant-based milk. In these products, dairy ingredients are partially or completely replaced with plant-based milk, such as soy, almond, hemp, rice, peanut, and others. This substitution contributes to the enrichment of products with bioactive compounds and enhances their sensory diversity, while most of the key chemical, physical, and textural properties remain comparable to those of plain dairy ice cream.

The analysis of nutritional and physicochemical properties of frozen desserts formulated with plant-based milk revealed a wide variation depending on product formulation and the processing methods used for the ingredients, particularly plant-based milk. At the same time, this analysis made it possible to identify the main groups of indicators influencing product quality, namely nutritional, chemical, physical, sensory, functional, and microbiological. This approach enabled the construction of a property tree for frozen dessert quality indicators and the development of a mathematical model based on it for evaluating the quality of such products. The proposed model is based on the use of weighting coefficients that reflect the importance of each group of indicators and individual indicators, and it also takes into account the recommended (optimal) values of each indicator by comparing them with the corresponding values of the product. The calculation of quality indices for different frozen desserts enables a comprehensive numerical comparison and the identification of the most preferable product. The model can be further expanded by incorporating additional quality indicators.

The SWOT analysis of frozen desserts containing plant-based milk made it possible to identify their strengths and weaknesses, as well as the associated opportunities and threats. This provided a basis for developing strategic directions for the advancement of such products, including the maximum utilization of local raw materials, particularly organic plant-based ingredients; enrichment with bioactive compounds to create functional products; diversification of sensory properties through the combination of different raw materials; and the application of advanced technologies and innovative ingredients to improve product quality and support brand positioning in terms of sustainability, health benefits, and ethical consumption.

Further research should focus on exploring the potential for combining different types of plant-based milk in frozen dessert formulations to achieve a balanced nutritional profile.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

Manuscript has no associated data.

Use of artificial intelligence statement

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

Authors' contributions

Igor Dudarev: Supervision, Conceptualization, Investigation, Data curation, Formal analysis, Resources, Validation, Writing – original draft.

Tamara Sydoruk: Conceptualization, Investigation, Data curation, Formal analysis, Visualization, Resources, Writing – original draft.

Mykola Andrushchenko: Conceptualization, Data curation, Formal analysis, Resources, Writing – original draft, Writing – review and editing.

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